

Form PTO 1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (REV 5-93)		ATTORNEY'S DOCKET NUMBER <b>B45139</b>
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED / ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) <b>09/647518</b>
INTERNATIONAL APPLICATION NO. <b>PCT/EP99/02278</b>	INTERNATIONAL FILING DATE <b>29 March 1999</b>	PRIORITY DATE CLAIMED <b>09 April 1998</b> <b>25 September 1998</b>
TITLE OF INVENTION <b>ADJUVANT COMPOSITIONS</b>		
APPLICANT(S) FOR DO/EO/US <b>Martin FRIEDE and Philippe HERMAND</b>		

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☒ has been transmitted by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

**Items 11. to 16. below concern other document(s) or information included:**


11. ☒ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.  
☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.  
☒ Please amend the specification by inserting before the first line the sentence: This is a 371 of International Application PCT/EP99/02278, filed 29 March 1999, which claims benefit from the following Provisional Applications: GB 9807805.8 filed September 4, 1998 and GB 9820956.2 filed September 25, 1998.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

US APPLICATION NO. (if known see 37 CFR 1.50)		INTERNATIONAL APPLICATION NO.		ATTORNEYS DOCKET NO.	
09/647518		PCT/EP99/02278		B45139	
17. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS PTO USE ONLY	
<b>Basic National Fee (37 C.F.R. 1.492(a)(1)-(5)):</b>					
Search Report has been prepared by the EPO or JPO .....\$840.00					
International Preliminary Examination Fee paid to USPTO (37 CFR 1.482) .....\$670.00					
No International Preliminary Examination Fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) .....\$690.00					
Neither International Preliminary Examination Fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....\$970.00					
International Preliminary Examination Fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....\$96.00					
<b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				<b>\$840.00</b>	
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				<b>\$0.00</b>	
Claims	Number Filed	Number Extra	Rate		
Total claims	30 - 20 =	10	10 x \$18.00	\$180.00	
Independent claims	8 - 3 =	8	8 x \$78.00	\$624.00	
Multiple dependent claims (if applicable)			+ \$260.00	\$260.00	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				<b>\$1,904.00</b>	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				\$	
<b>SUBTOTAL =</b>				<b>\$1,904.00</b>	
Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)) +				\$	
<b>TOTAL NATIONAL FEE =</b>				<b>\$1,904.00</b>	
				Amount to be refunded	\$
				charged	\$

- a. ☐ A check in the amount of \$\_\_\_\_\_ to cover the above fees is enclosed.
- b. ☒ Please charge my Deposit Account No. 19-2570 in the amount of **\$1,904.00** to cover the above fees.  
A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-2570. A duplicate copy of this sheet is enclosed.
- d. ☒ General Authorization to charge any and all fees under 37 CFR 1.16 or 1.17, including petitions for extension of time relating to this application (37 CFR 1.136 (a)(3)).

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.**

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09/647518

422 Rec'd PCT/PTO 29 SEP 2000

PATENT

ATTORNEY'S DOCKET NUMBER B45139

TRANSMITTAL LETTER TO THE U.S. DESIGNATED OFFICE  
(DO/US) - ENTRY INTO NATIONAL STAGE UNDER 35 USC 371

INTERNATIONAL APP. NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/02278	29 March 1999	09 April 1998
		25 September 1998

TITLE OF INVENTION  
ADJUVANT COMPOSITIONS


APPLICANT(S) FOR DO/US  
Martin FRIEDE and Philippe HERMAND

Box PCT  
Assistant Commissioner for Patents  
Washington, D.C. 20231  
ATTENTION: DO/US

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this Transmittal Letter, Form PTO 1390 and the papers indicated as being transmitted therewith, and Post Card are being deposited with the United States Postal Service on this date 29 September, 2000 in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EL231307675US addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Susan Galm  
(Typed or printed name of person mailing paper)

  
(Signature of person mailing paper)

422 Rec'd PCT/PTO 29 SEP 2000

**"EXPRESS MAIL CERTIFICATE"**  
**"EXPRESS MAIL" MAILING LABEL NUMBER EL231307675US**  
**DATE OF DEPOSIT 29 September 2000**

Attorney Docket No. B45139

IN THE UNITED STATES INTERNATIONAL EXAMINING AUTHORITY

Applicant: Friede, et al.

29 September 2000

International App. No.: PCT/EP99/02278

Group Art Unit No.: Unknown

International Filing Date: 29 March 1999

Examiner: Unknown

For: COMBINED VACCINE COMPOSITIONS

Assistant Commissioner of Patents

Box: PCT

Washington, D.C. 20231

**PRELIMINARY AMENDMENT**

Preliminary to the examination of this application, applicants respectfully request amendment of the above-identified application as follows:

**IN THE CLAIMS:**

Please cancel claims 25-29, 31 and 36, and please amend claims 3, 4, 6-8, 13, 14, 16, 19, 21, 22, 24, 32-35, as follows.

3. (Amended) An adjuvant composition as claimed in claim 1 or claim 2, wherein the adjuvant composition does not comprise an oil-in-water or water-in-oil emulsion.

4. (Amended) An adjuvant composition as claimed in [any one of claims 1 to 3] claim 1 or 2, characterised in that the surfactant of formula (1) is haemolytic.

6. (Amended) An adjuvant as claimed in claim 4 [or claim 5], wherein the surfactant of formula (1) has a haemolytic activity within a ten fold difference to that of polyoxyethylene-9 lauryl ether or polyoxyethylene-8 stearyl ether, as measured in the Guinea Pig blood haemolysis assay.

7. (Amended) An adjuvant composition as claimed in any one of claims 1 [to 6], 2 or 5, comprising a surfactant of formula (1), wherein  $n$  is 4 to 24.

8. (Amended) An adjuvant composition as claimed in any one of claims 1 [to 7], 2 or 5, comprising a surfactant of formula (1), wherein R is C<sub>8-20</sub> alkyl or Phenyl C<sub>8-20</sub> alkyl.

13. (Amended) An adjuvant composition as claimed in any one of claims 1 [to 12], 2, 5, 9, or 11, comprising a surfactant of formula (1), wherein A is a bond, thereby forming an ether.

14. (Amended) An adjuvant composition as claimed in any one of claims 1 [to 12], 2, 5, 9, or 11, comprising a surfactant of formula (1), wherein A is -C(O)-, thereby forming an ester.

16. (Amended) An adjuvant composition as claimed in any one of claims 1 [to 15], 2, 5, 9, or 11, wherein the concentration of the surfactant is in the range 0.1-10%.

19. (Amended) An adjuvant combination, comprising an adjuvant as claimed in any one of claims 1 [to 17], 2, 5, 9, 11, or 18, in combination with at least one additional immunostimulant.

21. (Amended) An adjuvant composition as claimed in any one of claims 1 [to 20], 2, 5, 9, 11, or 18, further comprising a vehicle, said vehicle comprising of any one of the following group: chitosan or other polycationic polymers, polylactide and polylactide-co-glycolide particles, particles composed of polysaccharides or chemically modified polysaccharides, or particles composed of glycerol monoesters.

22. (Amended) A vaccine composition comprising an adjuvant composition as claimed in any one of claims 1 [to 21], 2, 5, 9, 11, or 18, further comprising an antigen or antigenic composition.

24. (Amended) A vaccine as claimed in claim 22 [or 23] wherein the vaccine is in the form of an aerosol or spray.

32. (Amended) A method of treating a mammal suffering from or susceptible to a pathogenic infection, or cancer, or allergy, comprising the administration of a safe and effective amount of a vaccine composition according to [any of claims 22 to 24] claim 22 to the mammal.

Intl. App. No.: PCT/EP99/02278  
Docket No. B45139

33. (Amended) A method of treating a mammal suffering from or susceptible to a pathogenic infection or cancer or allergy, comprising the mucosal administration of a safe and effective amount of a vaccine composition according to [any of claims 22 to 24] claim 22.

34. (Amended) A method of treating a mammal suffering from or susceptible to a pathogenic infection or cancer or allergy, comprising the intranasal administration of a safe and effective amount of a vaccine composition according to [any of claims 22 to 24] claim 22.

35. (Amended) A process for making a vaccine composition according to [any one of claims 22 to 24] claim 22, comprising admixing (a) and adjuvant composition as claimed in any of claims 1 to 21, (b) a pharmaceutically acceptable excipient, and (c) an antigen or antigenic composition.

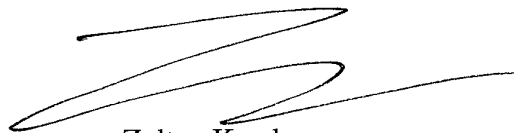
#### REMARKS

The above-identified application is being entered into the National Phase from PCT application no. PCT/EP99/02278.

Applicants have cancelled claims 25-29, 31 and 36, and amended claims 3, 4, 6-8, 13, 14, 16, 19, 21, 22, 24, 32-35, to put the claims in conformity with U.S. practice.

No new matter has been introduced.

Respectfully submitted,



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ADJUVANT COMPOSITIONS

- The present invention relates to an adjuvant composition comprising a polyoxyethylene ether or polyoxyethylene ester, in combination with a pharmaceutically acceptable excipient, and to a vaccine comprising such adjuvant compositions and antigen. In addition, the present invention relates to the use of polyoxyethylene ethers or esters in the manufacture of an adjuvant formulations, and vaccine formulations, and their use as medicaments.
- 10 Mucosal vaccination, for example intranasal and oral, may represent an easy and more convenient way of vaccination than traditional vaccination through systemic injection. The use of an injection to administer a vaccine dose is associated with a number of disadvantages, namely pain and irritation at the injection site following injection. These factors may lead to "needle-fear" which has been known to result in poor patient compliance for vaccination regimes. Furthermore, conventional systemic injections can be a source of infection in the region of the skin puncture.
- 15 Apart from bypassing the requirement for injection, mucosal vaccination is attractive since it has been shown in animals that mucosal administration of antigens has a greater efficiency of inducing protective responses at mucosal surfaces, which is the route of entry of many pathogens. In addition, it has been suggested that mucosal vaccination, such as intranasal vaccination, may induce mucosal immunity not only in the nasal mucosa, but also in distant mucosal sites such as the genital mucosa (Mestecky, 1987, Journal of Clinical Immunology, 7, 265-276; McGhee and Kiyono, Infectious Agents and Disease, 1993, 2, 55-73).
- 20 In order for mucosal immunisation to be a viable replacement for, or alternative to, immunisation through injection, this vaccination route will have to be able to induce systemic immunological responses at least as efficiently as those induced by injection. While it has been reported that certain antigens when administered via this route are able to induce systemic responses (Cahill *et al.*, 1993, FEMS Microbiology Letters,
- 25
- 30

107, 211-216), most soluble antigens given intranasally by themselves induce little or no immune response.

5 A number of authors have investigated potential mucosal adjuvants to overcome this problem, which exert their adjuvant activity through various mechanisms including: encapsulation of the antigen (*e.g.* liposomes and microparticles); or *via* direct interaction with, and subsequent release of immunostimulatory cytokines from, target cells (*e.g.* cholera toxin and *E.coli* heat-labile toxin); or by enhancing the uptake of antigen across the epithelium (*e.g.* cholera toxin).

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The applicant presents here the surprising finding that polyoxyethylene ethers and polyoxyethylene esters act as a potent adjuvants for vaccines. The adjuvants of the present invention are safe, easily sterilisable, and simple to administer.

15 Advantageously, such compositions are sufficient to induce systemic immune responses when administered mucosally, which are at least as high as those observed after conventional systemic injection of the vaccine.

Polyoxyethylene ethers such as polyoxyethylene lauryl ether are described in the Merck index (12<sup>th</sup> ed: entry 7717), where therapeutic uses are stated to include: topical  
20 anesthetic; anti-pruritic; and sclerosing agent activities. As a class, such polyoxyethylene ethers, or esters, are non-ionic surfactants.

Intranasal administration of polyoxyethylene ethers and esters have been described for the enhancement of insulin uptake in the nasal cavity (Hirai *et al.* 1981, International  
25 Journal of Pharmaceutics, 9, 165-172; Hirai *et al.* 1981, International Journal of Pharmaceutics, 9, 173-184).

Other non-ionic surfactants have been utilised in vaccine formulations. It has been reported that vaccine preparations comprising an admixture of either polyoxyethylene  
30 castor oil or caprylic/capric acid glycerides, with polyoxyethylene sorbitan monoesters, and an antigen, are capable of inducing systemic immune responses after



topical administration to a mucosal membrane (WO 9417827). This patent application discloses the combination of TWEEN20™ (polyoxyethylene sorbitan monoester) and Imwitor742™ (caprylic/capric acid glycerides), or a combination of TWEEN20™ and polyoxyethylene castor oil is able to enhance the systemic immune response following intranasal immunisation. Details of the effect of this formulation on the enhancement of the immune response towards intranasally administered antigens have also been described in the literature (Gizurarson *et al.* 1996. Vaccine Research, 5, 69-75; Aggerbeck *et al.* 1997. Vaccine, 15, 307-316).

Novasomes (US 5,147,725) are paucilamellar vesicular structures comprising Polyoxyethylene ethers and cholesterol encapsulate the antigen and are capable of adjuvanting the immune response to antigens after systemic administration.

Surfactants have also been formulated in such a way as to form non-ionic surfactant vesicles (commonly known as neosomes, WO 95/09651). Such vesicles, in the presence of cholesterol form lipid-bilayer vesicles which are capable of entrapping antigen within the inner aqueous phase or within the bilayer itself.

We present here the surprising finding that relatively low concentrations of polyoxyethylene ethers or esters are able to significantly enhance the systemic immune response towards co-administered antigens. Furthermore, when used in mucosal vaccine formulations, the boosting effect of these adjuvants raises the systemic immunological responses to a level equal or superior to that achieved by conventional systemic injection of the antigen. These molecules represent a class of adjuvants suitable for application in humans either for conventional systemic vaccine purposes, or to replace systemic vaccination by mucosal vaccination.

As many available vaccine adjuvants function because of antigen encapsulation, surprisingly the present invention functions as a potent vaccine adjuvants in the form of a non-vesicular solution or suspension. Thus, one embodiment of the present invention provides for an adjuvant formulation comprising a surfactant of formula (I),

which is present in the form of a non-vesicular solution or suspension. Another embodiment of the present invention takes the form of a vaccine adjuvant comprising a surfactant of formula (I), formulated in the absence of cholesterol.

- 5 Vaccines and adjuvant formulations of the present invention comprise molecules of general formula (I):



wherein,  $n$  is 1-50,  $A$  is a bond or  $-\text{C}(\text{O})-$ ,  $R$  is  $\text{C}_{1-50}$  alkyl or Phenyl  $\text{C}_{1-50}$  alkyl.

- 10 One embodiment of the present invention consists of a vaccine formulation comprising a polyoxyethylene ether of general formula (I), wherein  $n$  is between 1 and 50, preferably 4-24, most preferably 9; the  $R$  component is  $\text{C}_{1-50}$ , preferably  $\text{C}_4$ - $\text{C}_{20}$  alkyl and most preferably  $\text{C}_{12}$  alkyl, and  $A$  is a bond. The concentration of the polyoxyethylene ethers should be in the range 0.1-20%, preferably from 0.1-10%, and
- 15 most preferably in the range 0.1-1%. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-stearyl ether, polyoxyethylene-8-stearyl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.
- 20 A further embodiment of the present invention consists of a vaccine composition comprising a polyoxyethylene ester of general formula (I), wherein  $n$  is between 1 and 50, preferably 4-24, most preferably 9;  $R$  is  $\text{C}_{1-50}$ , preferably  $\text{C}_4$  to  $\text{C}_{20}$  alkyl and most preferably  $\text{C}_{12}$  alkyl, and  $A$  is  $-\text{C}(\text{O})-$ . The concentration of the polyoxyethylene ester should be in the range 0.1-20%, preferably from 0.1-10%, and most preferably in
- 25 the range 0.1-1%. Preferred polyoxyethylene esters are selected from the following group: polyoxyethylene-9-lauryl esters, polyoxyethylene-9-stearyl esters, polyoxyethylene-8-stearyl esters, polyoxyethylene-4-lauryl esters, polyoxyethylene-35-lauryl esters, and polyoxyethylene-23-lauryl esters.
- 30 Also forming an embodiment of the present invention are vaccine compositions comprising polyoxyethylene phenyl ethers of general formula (I), wherein  $n$  is

between 1 and 50 but preferably 4-24 and most preferably 9,  $R$  is  $C_{1-50}$  phenyl alkyl, preferably  $C_4 - C_{20}$  phenyl alkyl, and most preferably  $C_{12}$  phenyl alkyl, and  $A$  is a bond. The concentration of the polyoxyethylene ethers should preferably be in the range 0.1-10%, and most preferably in the range 0.25-1%.

5

The vaccine preparations of the present invention may be used to protect or treat a mammal susceptible to, or suffering from disease, by means of administering said vaccine via a mucosal route, such as the oral/buccal/intestinal/vaginal/rectal or nasal route. Such administration may be in a droplet, spray, or dry powdered form.

10

Nebulised or aerosolised vaccine formulations also form part of this invention.

Enteric formulations such as gastro resistant capsules and granules for oral administration, suppositories for rectal or vaginal administration also form part of this invention. The present invention may also be used to enhance the immunogenicity of antigens applied to the skin (transdermal or transcutaneous delivery). In addition, the

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adjuvants of the present invention may be parentally delivered, for example intramuscular, or subcutaneous administration, characterised in that the adjuvants are not in the form of a vesicle.

20

In a preferred embodiment of the present invention provides for an adjuvant for use in mucosal vaccine formulations. Such adjuvants are well tolerated in humans and are potent in their induction of systemic immune responses. The adjuvants of the present invention may take the form of a solution, or non-vesicular solution or suspension, and as such do not have any of the problems associated with the manufacture, stability, uniformity, and quality control of particulate adjuvant systems. These

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formulations are potent adjuvants and also exhibit low reactogenicity and are well tolerated by patients.

30

Preferably, the polyoxyethylene ethers of the present invention have haemolytic activity. The haemolytic activity of a polyoxyethylene ether may be measured *in vitro*, with reference to the following assay, and is as expressed as the highest concentration of the detergent which fails to cause lysis of the red blood cells:

1. Fresh blood from guinea pigs is washed with phosphate buffered saline (PBS) 3 times in a desk-top centrifuge. After resuspension to the original volume the blood is further diluted 10 fold in PBS.
- 5 2. 50 µl of this blood suspension is added to 800 µl of PBS containing two-fold dilutions of detergent.
3. After 8 hours the haemolysis is assessed visually or by measuring the optical density of the supernatant. The presence of a red supernatant, which absorbs light at 570 nm indicates the presence of haemolysis.
- 10 4. The results are expressed as the concentration of the first detergent dilution at which hemolysis no longer occurs.

Within the inherent experimental variability of such a biological assay, the polyoxyethylene ethers, or surfactants of general formula (I), of the present invention  
15 preferably have a haemolytic activity, of approximately between 0.5-0.0001%, more preferably between 0.05-0.0001%, even more preferably between 0.005-0.0001%, and most preferably between 0.003-0.0004%. Ideally, said polyoxyethylene ethers or esters should have a haemolytic activity similar (*i.e.* within a ten-fold difference) to that of either polyoxyethylene-9 lauryl ether or polyoxyethylene-8 stearyl ether.

20 The ratio of the length of the polyoxyethylene section to the length of the alkyl chain in the surfactant (*i.e.* the ratio of  $n$ : alkyl chain length), affects the solubility of this class of detergent in an aqueous medium. Thus, the adjuvants of the present invention may be in solution or may form particulate structures such as micelles. The adjuvants  
25 of the present invention are because of their non-vesicular nature are clear and not cloudy or opaque, stable and are easily sterilisable by filtration through a 220 nm membrane, and are manufactured in a easy and controlled fashion.

Vaccines of the present invention may take the form of a non-vesicular solution or  
30 suspension of polyoxyethylene ether or ester of general formula (I) in a pharmaceutically acceptable excipient, such as PBS or water, and an antigen or

antigenic preparation. Such a vaccine formulation may then be applied to a mucosal surface of a mammal in either a priming or boosting vaccination regime; or alternatively be administered systemically, for example *via* the transdermal, subcutaneous or intramuscular routes.

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Other adjuvants which are known to enhance both mucosal and systemic immunological responses include the bacterial enterotoxins derived from *Vibrio Cholerae* and *Eschericia Coli* (namely cholera toxin (CT), and heat-labile enterotoxin (LT) respectively). CT and LT are heterodimers consisting of a pentameric ring of  $\beta$ -subunits, cradling a toxic A subunit. Their structure and biological activity are disclosed in Clements and Finklestein, 1979, *Infection and Immunity*, 24:760-769; Clements et al., 1980, *Infection and Immunity*, 24:91-97. Recently a non-toxic derivative of LT has been developed which lacks the proteolytic site required to enable the non-toxic form of LT to be "switched on" into its toxic form, once released from the cell. This form of LT (termed mLT(R192G)) is rendered insuceptible to proteolytic cleavage by a substitution of the amino acid arginine with glycine at position 192, and has been shown to have a greatly reduced toxicity whilst retaining its potent adjuvant activity. mLT(R192G) is, therefore, termed a proteolytic site mutant. Methods for the manufacture of mLT(R192G) are disclosed in the patent application WO 96/06627. Other mutant forms of LT include the active site mutants such as mLT(A69G) which contain a substitution of an glycine for an alanine in position 69 of the LTA sequence. The use of mLT(R192G) as a mucosal vaccine is described in patent application WO 96/06627. Such adjuvants may be advantageously combined with the non-ionic surfactants of the present invention.

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Accordingly, in an alternative embodiment of the present invention the polyoxyethylene ether, or ester, will further be combined with other adjuvants or immunostimulants including Cholera toxin and its B subunit, Monophosphoryl Lipid A and its non-toxic derivative 3-de-O-acylated monophosphoryl lipid A (as described in UK patent no. GB 2,220,211), saponins such as Quil A (derived from the bark of the South American tree *Quillaja Saponaria Molina*), and fractions thereof, including

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QS21 and QS17 (US 5,057,540; Kensil, C. R., *Crit Rev Ther Drug Carrier Syst*, 1996, 12 (1-2):1-55; EP 0 362 279 B1; Kensil *et al.* (1991. *J. Immunology* vol 146, 431-437; WO 99/10008) and the oligonucleotide adjuvant system containing an unmethylated CpG dinucleotide (as described in WO 96/02555). A particularly preferred immunostimulant used in conjunction with POE is CpG immunostimulatory oligonucleotide, which formulations are potent in the induction and boosting of immune responses in larger animals. Preferred oligonucleotides have the following sequences: The sequences preferably contain all phosphorothioate modified internucleotide linkages.

10 OLIGO 1: TCC ATG ACG TTC CTG ACG TT

OLIGO 2: TCT CCC AGC GTG CGC CAT

OLIGO 3: ACC GAT GAC GTC GCC GGT GAC GGC ACC ACG

The CpG oligonucleotides utilised in the present invention may be synthesized by any method known in the art (eg EP 468520). Conveniently, such oligonucleotides may be synthesized utilising an automated synthesizer.

Alternatively polyoxyethylene ethers or esters may be combined with vaccine vehicles composed of chitosan or other polycationic polymers, polylactide and polylactide-co-glycolide particles, particles composed of polysaccharides or chemically modified polysaccharides, cholesterol-free liposomes and lipid-based particles, oil in water emulsions (WO 95/17210), particles composed of glycerol monoesters, *etc.* The polyoxyethylene ethers or esters may also be admixed with powdered excipients such as lactose containing antigen which can be administered as a dry powder.

25 Adjuvants of the present invention comprise the surfactants: polyoxyethylene ethers or esters wherein the polyoxyethylene ethers or esters are not present in the form of vesicles. Accordingly, the present invention includes the use of polyoxyethylene ethers and esters of general formula (I) in the manufacture of adjuvant compositions and vaccines, wherein the surfactant of general formula (I) is not present in a vesicular form.

- Preferably the vaccine formulations of the present invention contain an antigen or antigenic composition capable of eliciting an immune response against a human pathogen, which antigen or antigenic composition is derived from HIV-1, (such as tat, nef, gp120 or gp160), human herpes viruses, such as gD or derivatives thereof or
- 5 Immediate Early protein such as ICP27 from HSV1 or HSV2, cytomegalovirus ((esp Human)(such as gB or derivatives thereof), Rotavirus (including live-attenuated viruses), Epstein Barr virus (such as gp350 or derivatives thereof), Varicella Zoster Virus (such as gpI, II and IE63), or from a hepatitis virus such as hepatitis B virus (for example Hepatitis B Surface antigen or a derivative thereof), hepatitis A virus,
  - 10 hepatitis C virus and hepatitis E virus, or from other viral pathogens, such as paramyxoviruses: Respiratory Syncytial virus (such as F and G proteins or derivatives thereof), parainfluenza virus, measles virus, mumps virus, human papilloma viruses (for example HPV6, 11, 16, 18, ..), flaviviruses (e.g. Yellow Fever Virus, Dengue Virus, Tick-borne encephalitis virus, Japanese Encephalitis Virus) or Influenza virus
  - 15 (whole live or inactivated virus, split influenza virus, grown in eggs or MDCK cells, or Vero cells or whole flu virosomes (as described by R. Gluck, Vaccine, 1992, 10, 915-920) or purified or recombinant proteins thereof, such as HA, NP, NA, or M proteins, or combinations thereof), or derived from bacterial pathogens such as *Neisseria spp*, including *N. gonorrhea* and *N. meningitidis* (for example capsular
  - 20 polysaccharides and conjugates thereof, transferrin-binding proteins, lactoferrin binding proteins, PilC, adhesins); *S. pyogenes* (for example M proteins or fragments thereof, C5A protease, lipoteichoic acids), *S. agalactiae*, *S. mutans*; *H. ducreyi*; *Moraxella spp*, including *M. catarrhalis*, also known as *Branhamella catarrhalis* (for example high and low molecular weight adhesins and invasins); *Bordetella spp*,
  - 25 including *B. pertussis* (for example pertactin, pertussis toxin or derivatives thereof, filamentous hemagglutinin, adenylate cyclase, fimbriae), *B. parapertussis* and *B. bronchiseptica*; *Mycobacterium spp.*, including *M. tuberculosis* (for example ESAT6, Antigen 85A, -B or -C), *M. bovis*, *M. leprae*, *M. avium*, *M. paratuberculosis*, *M. smegmatis*; *Legionella spp*, including *L. pneumophila*; *Escherichia spp*, including
  - 30 enterotoxigenic *E. coli* (for example colonization factors, heat-labile toxin or derivatives thereof, heat-stable toxin or derivatives thereof), enterohemorrhagic *E. coli*,

- enteropathogenic *E. coli* (for example shiga toxin-like toxin or derivatives thereof); *Vibrio* spp, including *V. cholera* (for example cholera toxin or derivatives thereof); *Shigella* spp, including *S. sonnei*, *S. dysenteriae*, *S. flexnerii*; *Yersinia* spp, including *Y. enterocolitica* (for example a Yop protein), *Y. pestis*, *Y. pseudotuberculosis*;
- 5 *Campylobacter* spp, including *C. jejuni* (for example toxins, adhesins and invasins) and *C. coli*; *Salmonella* spp, including *S. typhi*, *S. paratyphi*, *S. choleraesuis*, *S. enteritidis*; *Listeria* spp., including *L. monocytogenes*; *Helicobacter* spp, including *H. pylori* (for example urease, catalase, vacuolating toxin); *Pseudomonas* spp, including *P. aeruginosa*; *Staphylococcus* spp., including *S. aureus*, *S. epidermidis*;
- 10 *Enterococcus* spp., including *E. faecalis*, *E. faecium*; *Clostridium* spp., including *C. tetani* (for example tetanus toxin and derivative thereof), *C. botulinum* (for example botulinum toxin and derivative thereof), *C. difficile* (for example clostridium toxins A or B and derivatives thereof); *Bacillus* spp., including *B. anthracis* (for example botulinum toxin and derivatives thereof); *Corynebacterium* spp., including *C.*
- 15 *diphtheriae* (for example diphtheria toxin and derivatives thereof); *Borrelia* spp., including *B. burgdorferi* (for example OspA, OspC, DbpA, DbpB), *B. garinii* (for example OspA, OspC, DbpA, DbpB), *B. afzelii* (for example OspA, OspC, DbpA, DbpB), *B. andersonii* (for example OspA, OspC, DbpA, DbpB), *B. hermsii*; *Ehrlichia* spp., including *E. equi* and the agent of the Human Granulocytic Ehrlichiosis;
- 20 *Rickettsia* spp, including *R. rickettsii*; *Chlamydia* spp., including *C. trachomatis* (for example MOMP, heparin-binding proteins), *C. pneumoniae* (for example MOMP, heparin-binding proteins), *C. psittaci*; *Leptospira* spp., including *L. interrogans*; *Treponema* spp., including *T. pallidum* (for example the rare outer membrane proteins), *T. denticola*, *T. hyodysenteriae*; or derived from parasites such as
- 25 *Plasmodium* spp., including *P. falciparum*; *Toxoplasma* spp., including *T. gondii* (for example SAG2, SAG3, Tg34); *Entamoeba* spp., including *E. histolytica*; *Babesia* spp., including *B. microti*; *Trypanosoma* spp., including *T. cruzi*; *Giardia* spp., including *G. lamblia*; *Leshmania* spp., including *L. major*; *Pneumocystis* spp., including *P. carinii*; *Trichomonas* spp., including *T. vaginalis*; *Schistosoma* spp., including *S.*
- 30 *mansoni*, or derived from yeast such as *Candida* spp., including *C. albicans*; *Cryptococcus* spp., including *C. neoformans*.



Preferred bacterial vaccines comprise antigens derived from *Streptococcus spp.*, including *S. pneumoniae* (for example capsular polysaccharides and conjugates thereof, PsaA, PspA, streptolysin, choline-binding proteins) and the protein antigen

5 Pneumolysin (Biochem Biophys Acta, 1989, 67, 1007; Rubins et al., Microbial Pathogenesis, 25, 337-342), and mutant detoxified derivatives thereof (WO 90/06951; WO 99/03884). Other preferred bacterial vaccines comprise antigens derived from *Haemophilus spp.*, including *H. influenzae type B* (for example PRP and conjugates thereof), *non typeable H. influenzae*, for example OMP26, high molecular weight

10 adhesins, P5, P6, protein D and lipoprotein D, and fimbrin and fimbrin derived peptides (US 5,843,464) or multiple copy variants or fusion proteins thereof. Other preferred bacterial vaccines comprise antigens derived from *Moraxella Catarrhalis* (including outer membrane vesicles thereof, and OMP106 (WO97/41731)) and from *Neisseria meningitidis B* (including outer membrane vesicles thereof, and NspA (WO

15 96/29412).

Derivatives of Hepatitis B Surface antigen are well known in the art and include, inter alia, those PreS1, PreS2 S antigens set forth described in European Patent applications EP-A-414 374; EP-A-0304 578, and EP 198-474. In one preferred aspect the vaccine

20 formulation of the invention comprises the HIV-1 antigen, gp120, especially when expressed in CHO cells. In a further embodiment, the vaccine formulation of the invention comprises gD2t as hereinabove defined.

In a preferred embodiment of the present invention vaccines containing the claimed

25 adjuvant comprise antigen derived from the Human Papilloma Virus (HPV) considered to be responsible for genital warts, (HPV 6 or HPV 11 and others), and the HPV viruses responsible for cervical cancer (HPV16, HPV18 and others).

Particularly preferred forms of genital wart prophylactic, or therapeutic, vaccine

30 comprise L1 particles or capsomers, and fusion proteins comprising one or more antigens selected from the HPV 6 and HPV 11 proteins E6, E7, L1, and L2.

The most preferred forms of fusion protein are: L2E7 as disclosed in WO 96/26277, and proteinD(1/3)-E7 disclosed in GB 9717953.5 (PCT/EP98/05285).

- 5 A preferred HPV cervical infection or cancer, prophylaxis or therapeutic vaccine, composition may comprise HPV 16 or 18 antigens. For example, L1 or L2 antigen monomers, or L1 or L2 antigens presented together as a virus like particle (VLP) or the L1 alone protein presented alone in a VLP or capsomer structure. Such antigens, virus like particles and capsomer are per se known. See for example WO94/00152,  
10 WO94/20137, WO94/05792, and WO93/02184.

Additional early proteins may be included alone or as fusion proteins such as preferably E7, E2 or E5 for example; particularly preferred embodiments of this includes a VLP comprising L1E7 fusion proteins (WO 96/11272).

- 15 Particularly preferred HPV 16 antigens comprise the early proteins E6 or E7 in fusion with a protein D carrier to form Protein D - E6 or E7 fusions from HPV 16, or combinations thereof; or combinations of E6 or E7 with L2 (WO 96/26277).
- 20 Alternatively the HPV 16 or 18 early proteins E6 and E7, may be presented in a single molecule, preferably a Protein D- E6/E7 fusion. Such vaccine may optionally contain either or both E6 and E7 proteins from HPV 18, preferably in the form of a Protein D - E6 or Protein D - E7 fusion protein or Protein D E6/E7 fusion protein.
- 25 The vaccine of the present invention may additionally comprise antigens from other HPV strains, preferably from strains HPV 6, 11, 31, 33, or 45.

- Vaccines of the present invention further comprise antigens derived from parasites that cause Malaria. For example, preferred antigens from *Plasmodia falciparum*  
30 include RTS,S and TRAP. RTS is a hybrid protein comprising substantially all the C-terminal portion of the circumsporozoite (CS) protein of *P.falciparum* linked via four

amino acids of the preS2 portion of Hepatitis B surface antigen to the surface (S) antigen of hepatitis B virus. Its full structure is disclosed in the International Patent Application No. PCT/EP92/02591, published under Number WO 93/10152 claiming priority from UK patent application No.9124390.7. When expressed in yeast RTS is produced as a lipoprotein particle, and when it is co-expressed with the S antigen from HBV it produces a mixed particle known as RTS,S. TRAP antigens are described in the International Patent Application No. PCT/GB89/00895, published under WO 90/01496. A preferred embodiment of the present invention is a Malaria vaccine wherein the antigenic preparation comprises a combination of the RTS,S and TRAP antigens. Other plasmodia antigens that are likely candidates to be components of a multistage Malaria vaccine are *P. falciparum* MSP1, AMA1, MSP3, EBA, GLURP, RAP1, RAP2, Sequestrin, PfEMP1, Pf332, LSA1, LSA3, STARP, SALSA, PfEXP1, Pfs25, Pfs28, PFS27/25, Pfs16, Pfs48/45, Pfs230 and their analogues in *Plasmodium* spp.

The formulations may also contain an anti-tumour antigen and be useful for the immunotherapeutic treatment cancers. For example, the adjuvant formulation finds utility with tumour rejection antigens such as those for prostate, breast, colorectal, lung, pancreatic, renal or melanoma cancers. Exemplary antigens include MAGE 1 and MAGE 3 or other MAGE antigens for the treatment of melanoma, PRAME, BAGE or GAGE (Robbins and Kawakami, 1996, Current Opinions in Immunology 8, pps 628-636; Van den Eynde et al., International Journal of Clinical & Laboratory Research (submitted 1997); Correale et al. (1997), Journal of the National Cancer Institute 89, p293. Indeed these antigens are expressed in a wide range of tumour types such as melanoma, lung carcinoma, sarcoma and bladder carcinoma. Other Tumor-Specific antigens are suitable for use with adjuvant of the present invention and include, but are not restricted to Prostate specific antigen (PSA) or Her-2/neu, KSA (GA733), MUC-1 and carcinoembryonic antigen (CEA). Accordingly in one aspect of the present invention there is provided a vaccine comprising an adjuvant composition according to the invention and a tumour rejection antigen.

Additionally said antigen may be a self peptide hormone such as whole length Gonadotrophin hormone releasing hormone (GnRH, WO 95/20600), a short 10 amino acid long peptide, in the treatment of many cancers, or in immunocastration.

- 5 It is foreseen that compositions of the present invention will be used to formulate vaccines containing antigens derived from *Borrelia sp.*. For example, antigens may include nucleic acid, pathogen derived antigen or antigenic preparations, recombinantly produced protein or peptides, and chimeric fusion proteins. In particular the antigen is OspA. The OspA may be a full mature protein in a lipidated  
10 form virtue of the host cell (E.Coli) termed (Lipo-OspA) or a non-lipidated derivative. Such non-lipidated derivatives include the non-lipidated NS1-OspA fusion protein which has the first 81 N-terminal amino acids of the non-structural protein (NS1) of the influenza virus, and the complete OspA protein, and another, MDP-OspA is a non-lipidated form of OspA carrying 3 additional N-terminal amino acids.

- 15 Vaccines of the present invention may be used for the prophylaxis or therapy of allergy. Such vaccines would comprise allergen specific (for example Der p1) and allergen non-specific antigens (for example peptides derived from human IgE, including but not restricted to the stanworth decapeptide (EP 0 477 231 B1)).

- 20 The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that each dose will  
25 comprise 1-1000 µg of protein, preferably 1-500 µg, preferably 1-100µg, most preferably 1 to 50µg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. Following an initial vaccination, subjects may receive one or several booster immunisation adequately spaced.

30

It is foreseen that compositions of the present invention will be used to formulate vaccines containing antigens derived from a wide variety of sources. For example, antigens may include human, bacterial, or viral nucleic acid, pathogen derived antigen or antigenic preparations, tumour derived antigen or antigenic preparations, host-  
5 derived antigens, including GnRH and IgE peptides, recombinantly produced protein or peptides, and chimeric fusion proteins.

The vaccines of the present invention may also be administered via the oral route. In such cases the pharmaceutically acceptable excipient may also include alkaline  
10 buffers, or enteric capsules or microgranules. The vaccines of the present invention may also be administered by the vaginal route. In such cases, the pharmaceutically acceptable excipients may also include emulsifiers, polymers such as CARBOPOL®, and other known stabilisers of vaginal creams and suppositories. The vaccines of the present invention may also be administered by the rectal route. In such cases the  
15 excipients may also include waxes and polymers known in the art for forming rectal suppositories.

The formulations of the present invention maybe used for both prophylactic and therapeutic purposes. Accordingly, the present invention provides for a method of  
20 treating a mammal susceptible to or suffering from an infectious disease or cancer, or allergy, or autoimmune disease. In a further aspect of the present invention there is provided a vaccine as herein described for use in medicine. Vaccine preparation is generally described in New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978.

25 The present invention relates to the use of polyoxyethylene ethers or esters of general formula (I) in the manufacture of an adjuvant formulation, comprising a surfactant of formula (I) and a pharmaceutically acceptable excipient. The present invention relates to the use of polyoxyethylene ethers or esters of general formula (I) in the  
30 manufacture of vaccine formulation, comprising a surfactant of formula (I) and a pharmaceutically acceptable excipient and an antigen. The present invention also

relates to the use of polyoxyethylene ethers or esters of general formula (I) in the manufacture of an adjuvant formulation or vaccine, as described above, wherein the formulation does not contain cholesterol. The present invention further provides the use of polyoxyethylene ethers or esters of general formula (I) in the manufacture of an adjuvant formulation or vaccine, as described above, wherein the formulation is a non-vesicular solution or suspension.

Examples of suitable pharmaceutically acceptable excipients include water, phosphate buffered saline, isotonic buffer solutions.

Alternative terms or names for polyoxyethylene lauryl ether are disclosed in the CAS registry. The CAS registry number of polyoxyethylene lauryl ether is:

CAS REGISTRY NUMBER: 9002-92-0

The present invention is illustrated by, but not restricted to, the following examples.

**Example 1, Techniques used to measure antigen specific antibody (Ab) responses .**

ELISA for the measurement of OspA-specific serum IgG:

Maxisorp Nunc immunoplates are coated overnight at 4°C with 50 µl/well of 1 µg/ml of antigen OspA diluted in PBS (in rows B to H of plate), or with 50 µl of 5 µg/ml purified goat anti-mouse Ig (Boehringer), in PBS (row A). Free sites on the plates were blocked (1 hour, 37°C) using saturation buffer : PBS containing 1%BSA, 0.1% polyoxyethylene sorbitan monolaurate (TWEEN 20), and 4% Normal Bovine Serum (NBS). Then, serial 2-fold dilutions (in saturation buffer, 50 µl/well) of IgG isotype mixture, diluted in saturation buffer (50 µl per well), was added as a standard curve (mixture of mouse monoclonal antibodies IgG1, IgG2a and IgG2b from Sigma, starting at 200 ng/ml and put in row A) and serum samples (starting at a 1/100 dilution and put in rows B to H) are incubated for 1hr 30mins at 37°C. The plates are then washed (×3) with washing buffer (PBS, 0.1% polyoxyethylene sorbitan monolaurate (TWEEN 20)). Then, biotinylated goat anti-mouse IgG (Amersham)

- diluted 1/5000 in saturation buffer are incubated (50 µl/well) for 1hr 30mins, at 37°C. After 3 washings, and subsequent addition of streptavidin-horseradish peroxidase conjugate (Amersham), plates are washed 5 times and incubated for 20 min at room temperature with 50 µl/well of revelation buffer (OPDA 0.4 mg/ml (Sigma) and H<sub>2</sub>O<sub>2</sub> 0.03% in 50mM pH 4.5 citrate buffer). Revelation is stopped by adding 50 µl/well H<sub>2</sub>SO<sub>4</sub> 2N. Optical densities are read at 492 and 630 nm by using Biorad 3550 immunoreader. Antibody titre are calculated by the 4 parameter mathematical method using SoftMaxPro software.
- 10 Anti-TT, anti-FHA and anti-influenza IgG titres were measured using a similar technique, by replacing the OspA coating antigen with either TT, FHA, or whole influenza antigen. TT was supplied by a commercially available source (Behring). FHA was produced and purified by methods described in EP 0 427 462 B. Whole influenza virus, inactivated with β-propiolactone (BPL), was supplied by SSD
- 15 GmBH (Dresden Germany).

ELISA for the measurement of *S. Pneumoniae* polysaccharide (PS14 and PS19)-specific serum IgG in mice:

- Maxisorp Nunc immunoplates are coated for 2 hours at 37°C with 100 µl/well of 5 µg/ml (PS14) or 20 µg/ml (PS19) antigen diluted in PBS. The plates are then washed (×3) with washing buffer (PBS, 0.1% polyoxyethylene sorbitan monolaurate (TWEEN 20)). Then, serial 2-fold dilutions (in PBS TWEEN 20, 100 µl per well) of PS14 or PS19-specific monoclonal Ab (mAb) IgG1 added as a standard curve (starting at 785 ng/ml for PS14 or 2040 ng/ml for PS19, and put in row A) and serum
- 20 samples (starting at a 1/20 dilution and put in rows B to H) are incubated for 30mins at 20°C under agitation. Before to be added and diluted on the plate, both mAb standards and serum samples are pre-incubated with Common Polysaccharides (CPS) for 1 hour at 37°C, in order to eliminate aspecific reactions. The plates are then washed (×3) with washing buffer (PBS TWEEN 20). Then, peroxydase-conjugated
- 25 goat anti-mouse IgG (Jackson) diluted 1/5000 in PBS TWEEN 20 are incubated (100 µl/well) for 30 min at 20°C under agitation. After 3 washings, plates are incubated for
- 30

15 min at room temperature with 100 µl/well of revelation buffer (OPDA 0.4 mg/ml (Sigma) and H<sub>2</sub>O<sub>2</sub> 0.03% in 50mM pH 4.5 citrate buffer). Revelation is stopped by adding 50 µl/well HCl 1N. Optical densities are read at 492 and 630 nm by using Biorad 3550 immunoreader. Antibody titre are calculated by the 4 parameter

5 mathematical method using SoftMaxPro software.

ELISA for the measurement of OspA-specific serum Ig Abs in monkeys:

Maxisorp Nunc immunoplates are coated overnight at 4°C with 50 µl/well of 1 µg/ml OspA diluted in PBS. Free sites on the plates are blocked (1 hour, 37°C) using

10 saturation buffer : PBS containing 1%BSA, 0.1% polyoxyethylene sorbitan monolaurate (TWEEN 20). Then, serial 2-fold dilutions (in saturation buffer, 50 µl per well) of a reference serum added as a standard curve (serum having a mid-point titer of 60000 ELISA Unit/ml, starting at 12 EU/ml and put in row A ) and serum samples (starting at a 1/100 dilution and put in rows B to H) are incubated for 1hr  
15 30mins at 37°C. The plates are then washed (×3) with washing buffer (PBS, 0.1% polyoxyethylene sorbitan monolaurate (TWEEN 20)). Then, biotinylated goat anti-human Ig (Amersham) diluted 1/3000 in saturation buffer are incubated (50 µl/well) for 1hr 30mins, at 37°C. After 3 washings, and subsequent addition of streptavidin-horseradish peroxidase conjugate (Amersham), plates are washed 5 times and  
20 incubated for 20 min at room temperature with 50 µl/well of revelation buffer (OPDA 0.4 mg/ml (Sigma) and H<sub>2</sub>O<sub>2</sub> 0.03% in 50mM pH 4.5 citrate buffer). Revelation is stopped by adding 50 µl/well H<sub>2</sub>SO<sub>4</sub> 2N. Optical densities are read at 492 and 630 nm by using Biorad 3550 immunoreader. Antibody titre are calculated by the 4 parameter mathematical method using SoftMaxPro software.

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Anti-influenza immunoglobulin titres were measured using a similar technique, by replacing the OspA coating antigen with whole influenza virus antigen, inactivated with β-propiolactone (BPL), supplied by SSD GmbH manufacturer (Dresden, Germany).

30



ELISA for the measurement of OspA-specific nasal IgA Abs in monkeys:

Maxisorp Nunc immunoplates are coated overnight at 4°C with 50 µl/well of 1 µg/ml antigen OspA diluted in PBS (in rows B to H of plate), or with 50 µl of 5 µg/ml purified goat anti-human IgA (Sigma), in PBS (row A). Free sites on the plates are blocked (1 hour, 37°C) using saturation buffer : PBS containing 1%BSA, 0.1% polyoxyethylene sorbitan monolaurate (TWEEN 20), and 4% Normal Bovine Serum (NBS). Then, serial 2-fold dilutions (in saturation buffer, 50 µl per well) of a reference secretion added as a standard curve (secretion having a mid-point titer of 3000 ELISA Unit/ml, starting at 30 EU/ml and put in row A ) and nasal swabs (starting at a 1/5 dilution and put in rows B to H) are incubated for 2hr at 22°C. The plates are then washed (×3) with washing buffer (PBS, 0.1% polyoxyethylene sorbitan monolaurate (TWEEN 20)). Then, biotinylated goat anti-human IgA (ICN) at 0.2 µg/ml in saturation buffer are incubated (50 µl/well) for 1hr 30mins, at 37°C. After 3 washings, and subsequent addition of streptavidin-horseradish peroxidase conjugate (Amersham), plates are washed 5 times and incubated for 10 min at room temperature with 50 µl/well of revelation buffer (TMB, Biorad). Revelation is stopped by adding 50 µl/well H<sub>2</sub>SO<sub>4</sub> 0.4N. Optical densities are read at 450 and 630 nm by using Biorad 3550 immunoreader. Antibody titre are calculated by the 4 parameter mathematical method using SoftMaxPro software. Samples are considered to be positive when their IgA titre exceed the cut-off of the assay (0.3 EU/ml).

Inhibition assay for the measurement of serum LA2-like Antibody titres to lipo-OspA

Antibody titres in the vaccinees were studied with respect to their LA2-like specificity. LA2 is a murine monoclonal antibody which recognizes a conformational OspA epitope at the surface of the bacteria and has been shown to be able to kill *B. burgdorferi* in vitro, as well as to protect mice against a challenge with laboratory-grown spirochete (Schaible UE et al. 1990. Proc Natl Acad Sci USA 87:3768-3772). Moreover, LA-2 mab has been shown to correlate with bactericidal antibodies, and studies on human sera showed also a good correlation between the total anti-OspA IgG titers and the LA-2 titers (as measured by ELISA).

- Maxisorp Nunc immunoplates are coated overnight at 4°C with 50 µl/well of 0.5µg/ml lipo OspA diluted in PBS. Free sites were blocked with saturation buffer for 1hr at 37°C with (100 µl/well of saturation buffer: PBS/ BSA 1%/ Tween 20 0.1%/ NBS 4%). Serial 2-fold dilutions of LA2 monoclonal Ab (mAb) starting at 4 µg/ml were diluted in saturation buffer (50 µl per well) to form a standard curve. Dilutions of serum samples from the vaccinees (starting at a 1/10 dilution) were also added and the plates incubated for 2hrs at 37°C. The plates were washed after incubation 3 times with PBS/ TWEEN 20 (0.1%). LA2 mAb-peroxidase conjugate (1/10,000) diluted in saturation buffer was added to each well (50 µl/well) and incubated for 1hr at 37°C. After 5 washings, plates are incubated for 20 min at room temperature (in darkness) with 50 µl/well of revelation buffer (OPDA 0.4 mg/ml and H<sub>2</sub>O<sub>2</sub> 0.03% in 50mM pH 4.5 citrate buffer). The reaction and colour formation was stopped with H<sub>2</sub>SO<sub>4</sub> 2N. Optical densities are read at 492 and 630 nm by using Biorad 3550 immunoreader. LA2-like Ab titers are calculated by the 4 parameter mathematical method using SoftMaxPro software. LA2-like antibody titres were determined by comparison with the standard curve.

### **Example 2** *Intranasal boosting of mice with OspA antigen*

- Female Balb/c mice (8 animals per group) aged 8 weeks were immunised intramuscularly with 1 µg of the antigen lipo-OspA on 50 µg alum. After 3 months the mice were boosted intranasally (under anesthesia) with 10 µl of solution (5 µl per nostril, delivered as droplets by pipette) containing either A: 5 µg lipo-OspA; B: 5 µg lipo-OspA in 36 % tween-20, 10% Imwitor 742 ; C: 5 µg lipo-OspA in 36 % tween-20; D: 5 µg lipo-OspA in 18% polyoxyethylene-9 lauryl ether. 14 days after the boost the sera were assayed for Abs against lipo-OspA by IgG and LA2 anti-OspA ELISA (see example 1). The results, see figure 1, indicate that lipo-OspA administered intranasally is able to boost the systemic lipo-OspA specific IgG titres. This boost is only marginally increased by the presence of tween-20 plus Imwitor 742 or tween-20 alone. Polyoxyethylene-9 lauryl ether, on the other hand,

induces a very significant boost. A similar pattern is observed for the LA2 response (see figure 2).

**Example 3** *Intranasal boosting of mice with OspA antigen*

5

Groups of mice were primed as described in example 2. The mice were then boosted (using the method described in example 2) with 5 µg lipo-OspA alone (group A and C) or in the presence of B: 1 % sodium taurocholic acid; D: 1% dodecyl-maltoside; E: 36% tween 20 or F: 18% polyoxyethylene-9 lauryl ether. Since the experiment with groups A and B was performed at a different moment to that with groups C,D,E and F they are separated on the figures below (see figure 3). It is clear that 1% sodium taurocholate does not significantly adjuvant the boost above that obtained with the antigen alone. Dodecyl-maltoside at 1%, or tween-20 at 36% provide a slight adjuvant effect, but only polyoxyethylene-9 lauryl ether provides a very significant enhancement of the IgG response. A similar effect is observed for the LA2 response (see figure 4).

**Example 4** *Intranasal boosting of mice – Dose range study*

- 20 In order to assess the concentration of polyoxyethylene-9 lauryl ether required to provide the nasal adjuvanticity observed in the previous examples, we performed a dose-range assay, and in order to show that this effect can be achieved using other polyoxyethylene ethers we investigated the use of polyoxyethylene-23 lauryl ether. Mice primed as in example 1 were boosted intranasally with 10 µl containing 5 µg of
- 25 lipo-OspA in either A: PBS; B: 1% polyoxyethylene-9 lauryl ether; C: 2% polyoxyethylene-9 lauryl ether; D: 5% polyoxyethylene-9 lauryl ether; E: 1% polyoxyethylene-23 lauryl ether or; F: 10 % polyoxyethylene-23 lauryl ether. 14 days after the boost the sera were analysed as in example 2.
- 30 Figures 5 and 6, below, show that concentrations of polyoxyethylene-9 lauryl ether as low as 1% show a very significant enhancement of the immune response.

Polyoxyethylene-23-lauryl ether also significantly enhances the intranasal boost response.

**Example 5** *Combination vaccine – intranasal boosting*

5

In order to assess the applicability of polyoxyethylene ethers to the enhancement of systemic immune responses after intranasal boosting, female balb/c mice were primed intra-muscularly with the commercial DTPa vaccine (Diphtheria, Tetanus, acellular Pertussis vaccine: INFANRIX™ SmithKline Beecham, Belgium). The mice were  
10 primed once intramuscularly with 2 X 50 µl injections corresponding to 20% of the human dose. Three months later the mice were boosted (as in example 2) intranasally with either tetanus toxoid (TT: 5 µg) or filamentous haemagglutinin (FHA: 5 µg) in A: PBS; B: 1% polyoxyethylene-9 lauryl ether; or; C: by intramuscular injection of the DTPa vaccine (2x50µl). 14 days after the boosting the sera were analysed for their  
15 TT and FHA specific IgG. The titres are shown in figures 7 and 8.

It is clear that for TT the protein by itself does not induce a significant boost, but polyoxyethylene-9 lauryl ether is able to significantly boost the immune response. Surprisingly, the response obtained by intranasal boosting in the presence of this adjuvants is greater than that obtained following intramuscular boosting of the  
20 immune response. The administration of FHA by itself, induces an immune response which is further significantly enhanced by addition of the polyoxyethylene-9 lauryl ether as an adjuvant.

**Example 6** *Intranasal boosting of AGMs*

25

Many adjuvants have been shown to work in small rodents, but to have no effect when tested in larger mammals. In order to assess whether polyoxyethylene ethers were able to exert an adjuvant effect on intranasal boosting when this was performed in larger species, African Green monkeys (AGMs: 4 animals per group) were primed  
30 intramuscularly with lipo-OspA (10 µg) on alum (500 µg) by intramuscular injection. 10 months later the animals were boosted intranasally with 200 µl (100 µl per nostril

administered under anesthesia with a bidose spray device from Pfeiffer GmBH, Germany) containing 60 µg lipo-OspA in either A: PBS; or B: 1% polyoxyethylene-9 lauryl ether. After 14 days the sera were tested for anti-OspA immunoglobulin, and LA2 titres. Figures 9 and 10, show the geometric mean titres for each of the groups. Group C consisting of 10 AGMs that had received both the priming and the boost by intramuscular injection of lipo-OspA on alum were assayed for anti-OspA immunoglobulin responses (geometric mean titres shown for LA2 titres only, figure 10).

Lipo-OspA alone was able to boost the systemic response when administered intranasally to monkeys, but this boost is very significantly enhanced by the addition of 1% polyoxyethylene 9 lauryl ether. Surprisingly, the titres obtained following intranasal boosting in the presence of polyoxyethylene 9 lauryl ether are also greater than those obtained following an intramuscular injection (group C).

15

**Example 7 *Intranasal priming and boosting of AGMs***

In the previous examples we demonstrated that polyoxyethylene ethers could adjuvant an intranasal boosting of the systemic response. In this example we examine whether naive animals can be primed and boosted by the nasal route to induce a systemic immune response. In addition, in order to investigate the applicability of these adjuvants to larger animals, this experiment was performed in African Green Monkeys (AGMs).

African Green Monkeys (3 animals per group) were primed and boosted intranasally with 60 µg of lipo-OspA delivered in 200 µl (100 µl per nostril delivered with a bidose spray-device from Pfeiffer GmBH, Germany) of A: PBS; B: 1% polyoxyethylene-9 lauryl ether. 14 days after the boosting the sera were assayed for their Osp-A specific immunoglobulin. Figure 11, shows that when Lipo-OspA is not adjuvanted, no systemic immune response can be detected following intranasal

priming and boosting. When polyoxyethylene-9 lauryl ether is used as an adjuvant, this vaccination schedule induced significant anti-OspA titres.

**Example 8, Intranasal adjuvant effect of CpG on the induction of systemic and nasal humoral immune responses to lipo OspA antigen in primates**

This model was designed to investigate the priming and boosting effect of polyoxyethylene-9 lauryl ether (POE-9LE), with and without additional immunostimulants, in a primate priming and boosting model. Serum and nasal immunoglobulin responses were measured. The immunostimulant used in this study was the CpG 1001 as described in example 9.

*Experimental procedure*

African Green monkeys were primed and boosted intranasally at days 0 (pI) and 14 (pII). Vaccines were given using a bi-dose spray delivery system from the Pfeiffer company (100 µl in each nostril, under anesthesia). Formulations tested were:

Group	Antigen	Adjuvant	n=	Route
1	LipoOspA (60µg)	None	2	i.n.
2	lipoOspA (60µg)	CpG (100µg)	3	i.n.
3	lipoOspA (60µg)	CpG (100µg), POE-9 LE (0.25%)	3	i.n.
4	lipoOspA (60µg)	POE (0.25%)	4	i.n.
5	lipoOspA (60µg)	POE (0.5%)	4	i.n.

Ig Ab titers to lipo OspA were measured in sera collected at day 14 post-pII. Antigen-specific nasal IgA were measured using a very sensitive ELISA in nasal swabs collected at the same time, animals were considered positive when their IgA titres exceeded a pre-determined level which was significantly above background levels.

Results

*Serum OspA-specific immunoglobulin*

Figure 12 shows the levels of serum anti-lipo-OspA immunoglobulin responses observed at day 14 post-pII. Lipo-OspA given as a priming and boosting formulation alone did not induce any detectable serum immunoglobulin. This response was not improved in the presence of CpG. A dose of 0.25 % and 0.5 % of POE-9 LE elicited greater immune responses than those observed after vaccination with CpG alone, although the 0.5% dose is much more efficient in this respect. However, when combined with CpG, the 0.25 % dose induces an Ab response similar in magnitude to that obtained with 0.5 % dose, indicating a synergistic effect of the CpG and POE components.

#### *Nasal OspA-specific IgA*

As observed for the serum Ig response, vaccines containing lipo OspA alone or combined with CpG are unable to elicit detectable nasal IgA Abs (see figure 13 for a summary of all nasal responses). Only 25% animals given lipo OspA in combination with 0.25% polyoxyethylene lauryl ether were found to be "nasal IgA" positive (versus 50% in the 0.5% POE-9 LE). When CpG is added to this 0.25% POE formulation, 100% animals develop an IgA response. Therefore, a synergy between CpG and polyoxyethylene lauryl ether is also obtained for the induction of mucosal antibodies.

Thus, a synergy between polyoxyethylene lauryl ether and CpG is obtained in monkeys for the induction of antigen specific serum immunoglobulins and nasal IgA.

#### **Example 9, Intranasal adjuvant effect of CpG on the boosting of systemic humoral immune responses to lipo OspA antigen**

The following example was designed to investigate the effect of the addition of other immunostimulants into the polyoxyethylene ether (POE-9 LE) adjuvant system in a murine booster model. CpG is a known immunomodulatory oligonucleotide described in PCT WO 96/02555. The immune response boosted by these vaccine formulations

were at least as high as those induced by conventional i.m. boosting vaccinations. The formulations were further compared to a well known intranasal adjuvant, the heat-labile enterotoxin from *E. Coli* (mLT).

- 5 The CpG sequences used in this experiment were CpG 1001 (TCC ATG AGC TTC CTG ACG TT), CpG 1002 (TCT CCC AGC GTG CGC CAT), and the negative control the non-immunostimulatory sequence CpG1005 (TCC ATG AGC TTC CTG AGC TT).

10 *Experimental procedure*

Balb/c mice were primed at day 0 by intramuscular administration of 100 µl vaccine containing 1 µg lipo OspA adsorbed on 50 µg aluminium hydroxyde. At day 107, intranasal booster was given in 10 µl (5 µl in each nostril), by nasal drop administration with a micropipette under anesthesia. Groups of 6 mice were boosted

- 15 either intranasally (i.n.) or intramuscularly (i.m.) with the following vaccine formulations:

<i>Group</i>	<i>Antigen</i>	<i>Adjuvant</i>	<i>Route</i>
1	LipoOspA (5µg)	AlOH <sub>3</sub> (50µg)	i.m.
2	LipoOspA (5µg)	CpG1005 (20µg), POE-9 LE (1%)	i.n.
3	LipoOspA (5µg)	CpG1002 (20µg), POE-9 LE (1%)	i.n.
4	LipoOspA (5µg)	CpG1001 (20µg), POE-9 LE (1%)	i.n.
5	LipoOspA (5µg)	CpG1005 (20µg)	i.n.
6	LipoOspA (5µg)	CpG1002 (20µg)	i.n.
7	LipoOspA (5µg)	CpG1001 (20µg)	i.n.
8	LipoOspA (5µg)	POE-9 LE (1%)	i.n.
9	LipoOspA (5µg)	mLT (5µg)	i.n.
10	LipoOspA (5µg)	None	i.n.
11	Unboosted		



Bleedings were performed the day of boosting, and 14 days after the boost (pII). Specific serum IgG titers to OspA and LA2 titers were determined by ELISA on individual sera.

## 5 *Results*

As shown in figure 14 (showing OspA specific serum IgG as measured by antigen specific ELISA), and figure 15 (showing bacteriocidal LA2 titres in serum), no improvement of the serum OspA-specific Ab responses was imparted by CpG alone. The formulation of OspA with polyoxyethylene lauryl ether enhanced the resultant  
10 IgG and LA2 titers. The best responses were observed when lipo-OspA was formulated with both polyoxyethylene lauryl ether and CpG.

### **Example 10** *Dose study*

15 As described in the example 4, concentrations of polyoxyethylene-9 lauryl ether as low as 1% show a very significant enhancement of the immune response. In order to assess the concentration of polyoxyethylene-9 lauryl ether required to provide the nasal adjuvanticity observed in the previous examples, a dose-range assay with lower doses was performed.

20 Balb/c mice primed as in example 2 were boosted intranasally with 10  $\mu$ l containing 5  $\mu$ g of lipo-OspA in either A: PBS; B: 1% polyoxyethylene-9 lauryl ether; C: 0.5% polyoxyethylene-9 lauryl ether; D: 0.25% polyoxyethylene-9 lauryl ether; or; E: by intramuscular injection of 1  $\mu$ g lipo-OspA adsorbed on 50  $\mu$ g Alum. 14 days after the  
25 boost the sera were analyzed as in example 1.

### *Results*

Figures 16 and 17, below, show that concentrations of polyoxyethylene-9 lauryl ether  
30 as low as 0.25% show a very significant enhancement of the immune response. Even

with such a low dose of adjuvant, the Ab response reached is similar to that elicited by the parenteral vaccine.

**Example 11, *Anti-influenza vaccination in mice***

5

In order to assess the applicability of polyoxyethylene ethers to the enhancement of systemic anti-influenza immune responses after intranasal boosting, female Balb/c mice were primed intra-muscularly with classical monovalent split influenza vaccine. The mice were primed twice intramuscularly at days 0 and 14 with 100 µl injections containing 1.5 µg equivalent hemagglutinin A (HA) of A/Singapore/6/86 split monobulk. Three months later the mice were boosted (as in example 2) intranasally with 1.5 µg equivalent HA of inactivated whole A/Singapore/6/86 virus in A: PBS; B: 1% polyoxyethylene-9 lauryl ether; or; C: by intramuscular injection of the split A/Singapore/6/86 vaccine (1.5 µg equivalent HA). 14 days after the boosting the sera were analyzed for their A/Singapore/6/86 virus-specific IgG.

**Results**

The titres are shown in figure 18. It is clear that the plain antigen by itself does not induce a significant boost, but polyoxyethylene-9 lauryl ether is able to significantly boost the immune response. The Ab titres reached in the presence of this adjuvant are not significantly lower than those elicited by the parenteral vaccine.

**Example 12 *Anti-influenza vaccination in monkeys***

25

In the example 11, we demonstrated that polyoxyethylene-9 lauryl ether enhanced the immunogenicity of influenza antigen in mice. In order to assess whether this surfactant was able to exert a similar adjuvant effect in larger species, African Green monkeys (AGMs: 2 animals per group and per blood collection day) were primed and boosted intranasally (as in example 6) with 50 µg equivalent HA of inactivated whole A/Beijing/262/95 virus in 200 µl of A: PBS; B: 0.5% polyoxyethylene-9 lauryl ether.

At days 2, 7 and 14 after the boosting the sera were assayed for their A/Beijing/262/95 virus-specific Ig Abs. Figure 19 shows clearly that when polyoxyethylene-9 lauryl ether is used as an adjuvant, the immune response to influenza antigen is improved.

5    **Example 13**    *Vaccination studies with polysaccharide antigens*

The preceding examples demonstrate the ability of polyoxyethylene-9 lauryl ether to adjuvant the immune responses elicited to protein-type antigens. In this example, we examine whether this adjuvant is able to enhance the boosting effect of nasally-delivered polysaccharide antigens in mice primed parenterally. The mice were primed  
10    once subcutaneously with 100 µl injections containing *S. pneumoniae* PS14 and PS19 polysaccharides (1 µg each one) conjugated to the protein D carrier. Two months later, the mice were boosted intranasally (under anesthesia) with 40 µl of solution (10 µl per nostril at time 0 followed 30 minutes later by 10 µl per nostril again, delivered as droplets by pipette) containing 1 µg PS14 and 1 µg PS19 conjugates in either A:  
15    NaCl 150 mM pH 6.1; B: 1% polyoxyethylene-9 lauryl ether. 14 days after the boost the sera were assayed for their PS14 and PS19-specific IgG Abs.

*Results*

20    As shown in figures 20 and 21, the administration of PS14 or PS19 by itself induces a boosting response which is further enhanced by addition of polyoxyethylene-9 lauryl ether as an adjuvant.

**Example 14**    *Polyoxyethylene-8 stearyl ether*

25

In order to show that the adjuvant effect of polyoxyethylene-9 lauryl ether can be achieved using other polyoxyethylene ethers we investigated the use of polyoxyethylene-8 stearyl ether.

30    Balb/c mice primed as in example 2 were boosted intranasally with 10 µl containing 5 µg of lipo-OspA in either A: PBS; B: 1% polyoxyethylene-9 lauryl ether; C: 1%

polyoxyethylene-8 stearyl ether; or, D: by intramuscular injection of 1 µg lipo-OspA adsorbed on 50 µg Alum. 14 days after the boost the sera were analyzed as in example 1.

## 5 *Results*

Figures 22 and 23 show that polyoxyethylene-8 stearyl ether is as potent as polyoxyethylene-9 lauryl ether for enhancing the boosting response to the antigen. Ab titres reached with both polyoxyethylene ethers are similar to those elicited by the  
10 parenteral vaccine.

## ART 34 AMDT

## Claims (19/5/2000)

1. An adjuvant composition comprising a surfactant of formula (I):



wherein,  $n$  is 1-50,  $A$  is a bond or  $-\text{C}(\text{O})-$ ,  $R$  is  $\text{C}_{1-50}$  alkyl or Phenyl  $\text{C}_{1-50}$  alkyl;  
and a pharmaceutically acceptable excipient, characterised in that the surfactant of formula (I) is in the form of an aqueous solution or a micelle.

2. An adjuvant composition comprising a surfactant of formula (I):



wherein,  $n$  is 1-50,  $A$  is a bond or  $-\text{C}(\text{O})-$ ,  $R$  is  $\text{C}_{1-50}$  alkyl or Phenyl  $\text{C}_{1-50}$  alkyl;  
and a pharmaceutically acceptable excipient, characterised in that the surfactant of formula (I) is not in the form of a vesicle and also in that the adjuvant composition does not comprise an acrylic acid polymer.

3. An adjuvant composition as claimed in claim 1 or claim 2, wherein the adjuvant composition does not comprise an oil-in-water or water-in-oil emulsion.

4. An adjuvant composition as claimed in any one of claims 1 to 3, characterised in that the surfactant of formula (I) is haemolytic.

5. An adjuvant composition comprising a surfactant of formula (I):



wherein,  $n$  is 1-50,  $A$  is a bond or  $-\text{C}(\text{O})-$ ,  $R$  is  $\text{C}_{1-50}$  alkyl or Phenyl  $\text{C}_{1-50}$  alkyl;  
and a pharmaceutically acceptable excipient, characterised in that the surfactant of formula (I) is not in the form of a vesicle and also in that the degree of haemolytic activity is in the range of 0.05-0.0001% as measured in the Guinea Pig blood haemolysis assay.

6. An adjuvant as claimed in claim 4 or claim 5, wherein the surfactant of formula (I) has a haemolytic activity within a ten fold difference to that of polyoxyethylene-9 lauryl ether or polyoxyethylene-8 stearyl ether, as measured in the Guinea Pig blood haemolysis assay.

7. An adjuvant composition as claimed in any one of claims 1 to 6, comprising a surfactant of formula (I), wherein  $n$  is 4 to 24.

8. An adjuvant composition as claimed in any one of claims 1 to 7, comprising a surfactant of formula (I), wherein  $R$  is  $\text{C}_{8-20}$  alkyl or Phenyl  $\text{C}_{8-20}$  alkyl.

9. An adjuvant composition comprising a surfactant of formula (I):



wherein,  $n$  is 9,  $A$  is a bond or  $-\text{C}(\text{O})-$ ,  $R$  is  $\text{C}_{1-50}$  alkyl or Phenyl  $\text{C}_{1-50}$  alkyl;  
and a pharmaceutically acceptable excipient, characterised in that the surfactant of  
formula (I) is not in the form of a vesicle.

10. An adjuvant composition as claimed in claim 8, wherein  $R$  is  $\text{C}_{12}$  alkyl.

11. An adjuvant composition comprising a surfactant of formula (I):



wherein,  $n$  is 8,  $A$  is a bond or  $-\text{C}(\text{O})-$ ,  $R$  is  $\text{C}_{1-50}$  alkyl or Phenyl  $\text{C}_{1-50}$  alkyl;  
and a pharmaceutically acceptable excipient, characterised in that the surfactant of  
formula (I) is not in the form of a vesicle.

12. An adjuvant composition as claimed in claim 11, wherein  $R$  is  $\text{C}_{18}$  alkyl.

13. An adjuvant composition as claimed in any one of claims 1 to 12, comprising  
a surfactant of formula (I), wherein  $A$  is a bond, thereby forming an ether.

14. An adjuvant composition as claimed in any one of claims 1 to 12, comprising  
a surfactant of formula (I), wherein  $A$  is  $-\text{C}(\text{O})-$ , thereby forming an ester.

15. An adjuvant composition as claimed in claim 1, wherein the polyoxyethylene  
ether or ester of formula (I) is selected from the group comprising: polyoxyethylene-  
9-lauryl ether, polyoxyethylene-9-lauryl ester, polyoxyethylene-9-stearyl ether,  
polyoxyethylene-8-stearyl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-  
lauryl ether, polyoxyethylene-23-lauryl ether.

16. An adjuvant composition as claimed in any one of claims 1 to 15, wherein the  
concentration of the surfactant is in the range 0.1-10%.

17. An adjuvant composition as claimed in claim 16, wherein the concentration of  
the surfactant is in the range 0.25-1%.

18. An adjuvant combination, comprising an adjuvant comprising a surfactant of  
formula (I):



wherein,  $n$  is 1-50,  $A$  is a bond or  $-\text{C}(\text{O})-$ ,  $R$  is  $\text{C}_{1-50}$  alkyl or Phenyl  $\text{C}_{1-50}$  alkyl;  
and a pharmaceutically acceptable excipient, characterised in that the surfactant of  
formula (I) is not in the form of a vesicle; and at least one additional immunostimulant  
selected from the group comprising: LT, CT, 3D-MPL, CpG, and QS21.

19. An adjuvant combination, comprising an adjuvant as claimed in any one of claims 1 to 17, in combination with at least one additional immunostimulant.
20. An adjuvant combination as claimed in claim 19, wherein the at least one additional immunostimulant is selected from the group comprising: LT, CT, 3D-MPL, CpG, and QS21.
20. An adjuvant composition as claimed in claim 19, wherein the CpG adjuvant is: TCC ATG ACG TTC CTG ACG TT (SEQ ID NO. 1).
21. An adjuvant composition as claimed in any one of claim 1 to 20, further comprising a vehicle, said vehicle comprising of any one of the following group: chitosan or other polycationic polymers; polylactide and polylactide-co-glycolide particles, particles composed of polysaccharides or chemically modified polysaccharides, or particles composed of glycerol monoesters.
22. A vaccine composition comprising an adjuvant composition as claimed in any one of claims 1 to 21, further comprising an antigen or antigenic composition.
23. A vaccine as claimed in claim 22, wherein the antigen or antigen composition is derived from the group comprising: Human Immunodeficiency Virus, Varicella Zoster virus, Herpes Simplex Virus type 1, Herpes Simplex virus type 2, Human cytomegalovirus, Dengue virus, Hepatitis A, B, C or E, Respiratory Syncytial virus, human papilloma virus, Influenza virus, Hib, Meningitis virus, Salmonella, Neisseria, Borrelia, Chlamydia, Bordetella, Streptococcus, Mycoplasma, Mycobacteria, Haemophilus, Plasmodium or Toxoplasma, IgE peptides such as the stanworth decapeptide; or Tumor associated antigens (TMA), MAGE, BAGE, GAGE, MUC-1, Her-2 neu, LHRH, CEA, PSA, KSA, or PRAME.
24. A vaccine as claimed in claim 22 or 23, wherein the vaccine is in the form of an aerosol or spray.
25. Use of a surfactant of general formula (I):  

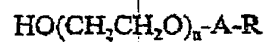
$$\text{HO}(\text{CH}_2\text{CH}_2\text{O})_n\text{-A-R}$$
 wherein, n is 1-50, A is a bond or -C(O)-, R is  $\text{C}_{1-50}$  alkyl or Phenyl  $\text{C}_{1-50}$  alkyl, wherein the surfactant of formula (I) is not in the form of a vesicle; in the manufacture of a medicament for application onto a mucosal surface or the skin of a patient.

26. Use of an adjuvant composition as claimed in any one of claims 1 to 21, in the manufacture of a medicament for application onto a mucosal surface or the skin of a patient.
27. Use of a surfactant of general formula (I) as claimed in claim 25, characterised in that the final medicament does not comprise an acrylic acid polymer.
28. Use of a surfactant of general formula (I) as claimed in claim 25, characterised in that the final medicament does not comprise an oil-in-water or water-in-oil emulsion.
29. Use of polyoxyethylene-9 lauryl ether or polyoxyethylene-8 stearyl ether in the manufacture of a medicament for application onto a mucosal surface of a patient.
30. A spray device, more particularly a bi-dose spray device, filled with a vaccine, characterised in that the vaccine comprises: (a) a surfactant of general formula (I):  $\text{HO}(\text{CH}_2\text{CH}_2\text{O})_n\text{-A-R}$  wherein, n is 1-50, A is a bond or  $-\text{C}(\text{O})-$ , R is  $\text{C}_{1-50}$  alkyl or Phenyl  $\text{C}_{1-50}$  alkyl; (b) a pharmaceutically acceptable excipient; and (c) an antigen or antigenic composition.
31. Use of vaccine composition as defined in any of claims 22 to 24, for the manufacture of a vaccine for the treatment of viral, bacterial, parasitic infections, allergy, or cancer.
32. A method of treating a mammal suffering from or susceptible to a pathogenic infection, or cancer, or allergy, comprising the administration of a safe and effective amount of a vaccine composition according to any of claims 22 to 24 to the mammal.
33. A method of treating a mammal suffering from or susceptible to a pathogenic infection, or cancer, or allergy, comprising the mucosal administration of a safe and effective amount of a vaccine composition according to any of claims 22 to 24.
34. A method of treating a mammal suffering from or susceptible to a pathogenic infection, or cancer, or allergy, comprising the intranasal administration of a safe and effective amount of a vaccine composition according to any of claims 22 to 24.
35. A process for making a vaccine composition according to any one of claims 22 to 24, comprising admixing (a) an adjuvant composition as claimed in any one of claims 1 to 21, (b) a pharmaceutically acceptable excipient, and (c) an antigen or antigenic composition.



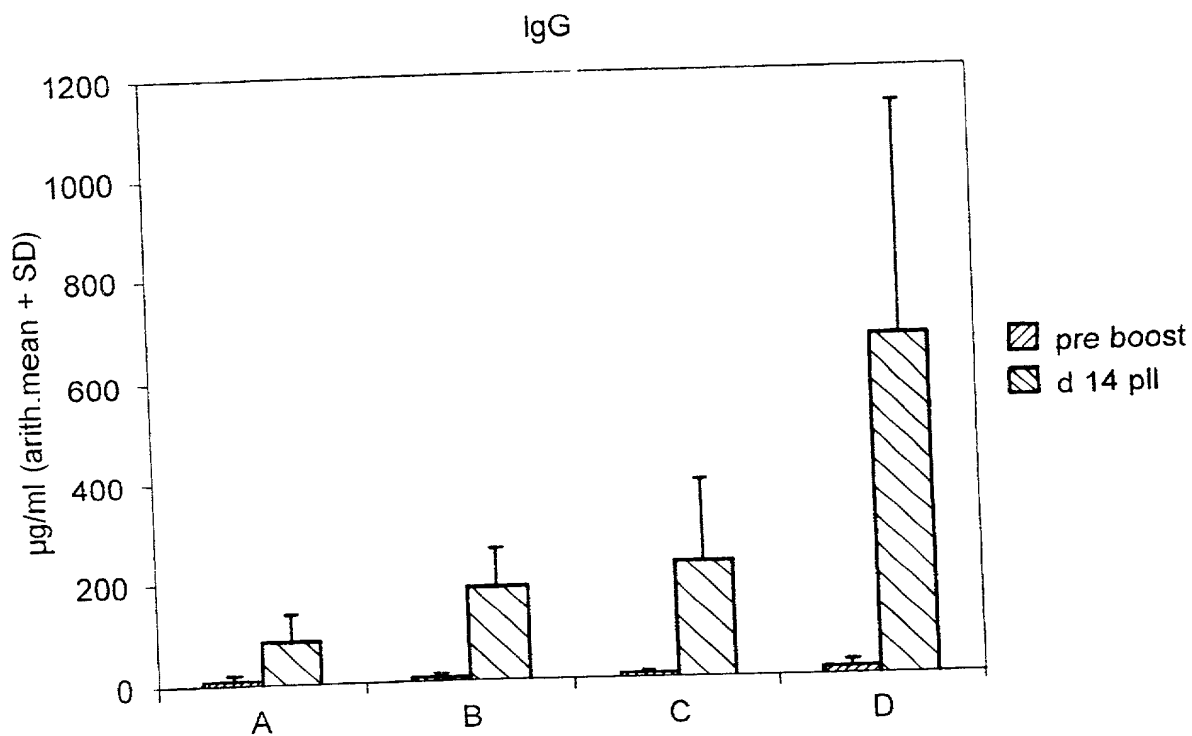
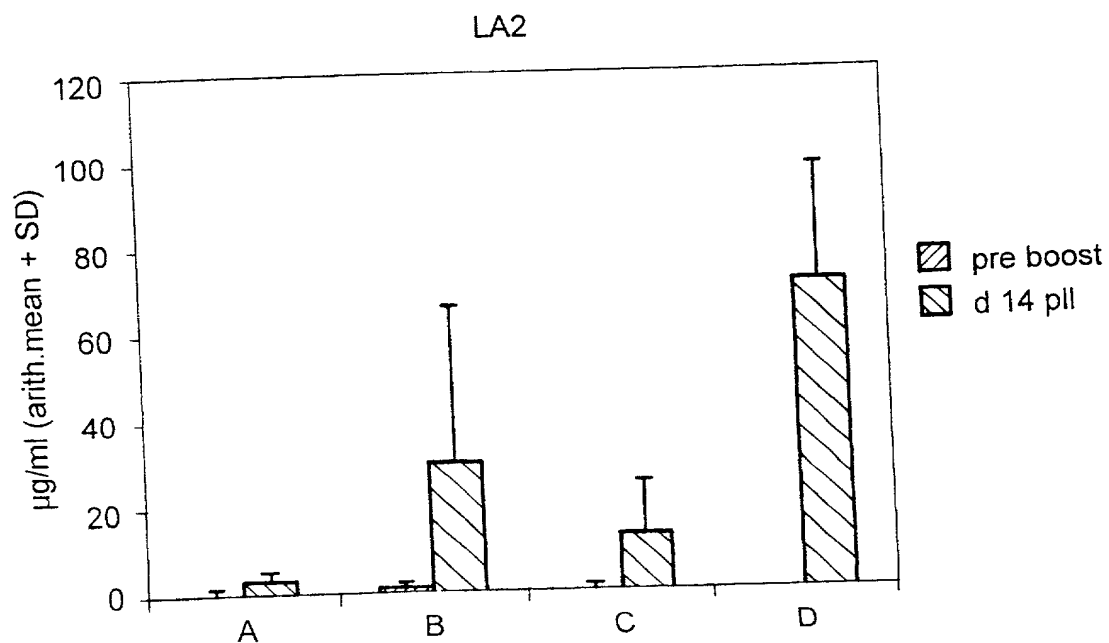
36. A vaccine or adjuvant as claimed in any one of claims 1 to 24; for use as a medicament.

37. An vaccine composition comprising an adjuvant composition, a pharmaceutically acceptable excipient and an antigen or antigenic composition; wherein the adjuvant composition consists of a surfactant of formula (I):

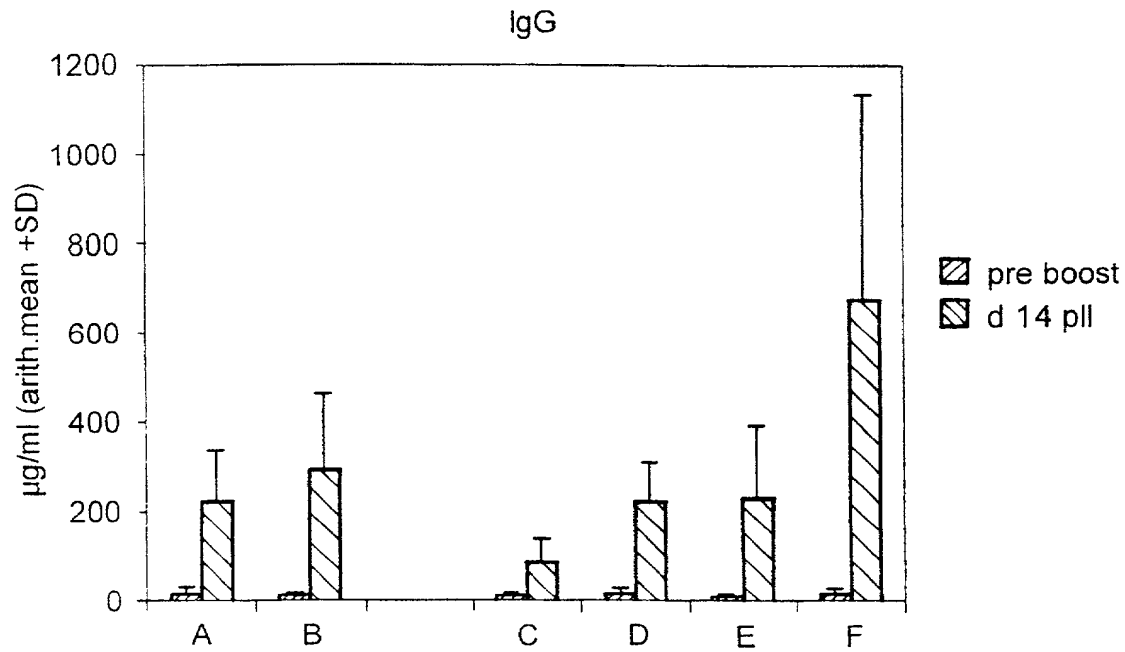


wherein,  $n$  is 1-50,  $A$  is a bond or  $-\text{C}(\text{O})-$ ,  $R$  is  $\text{C}_{1-50}$  alkyl or Phenyl  $\text{C}_{1-50}$  alkyl.

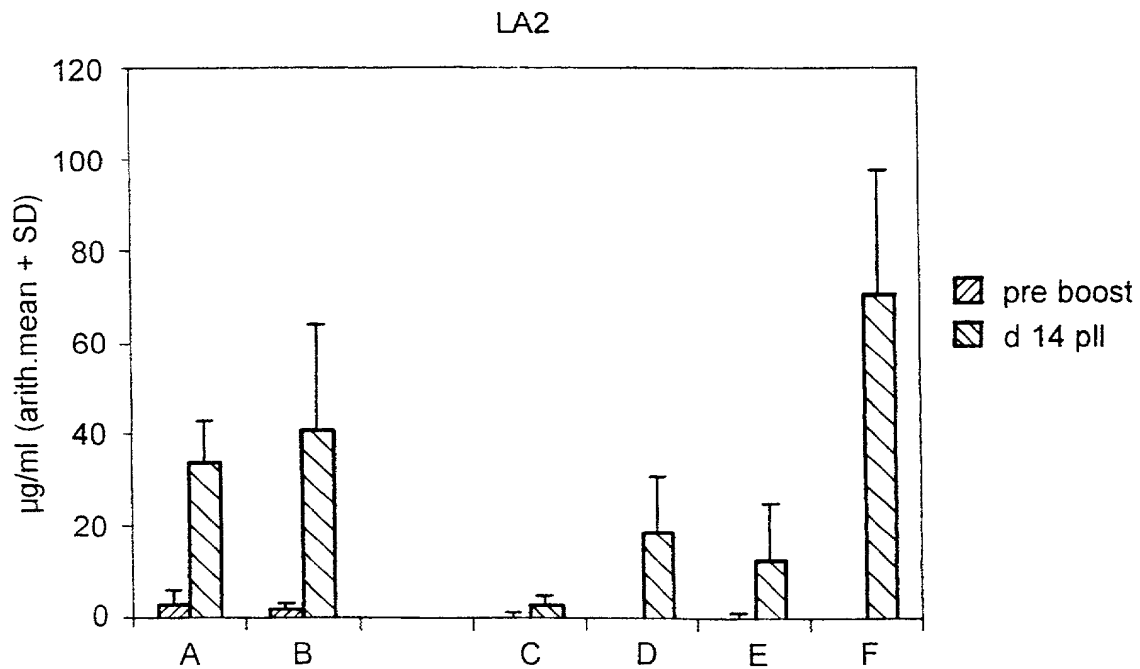
1/12

**Fig. 1** Anti-OspA responses in mice (see example 2)**Fig. 2** LA2 titres in mice (see example 2)

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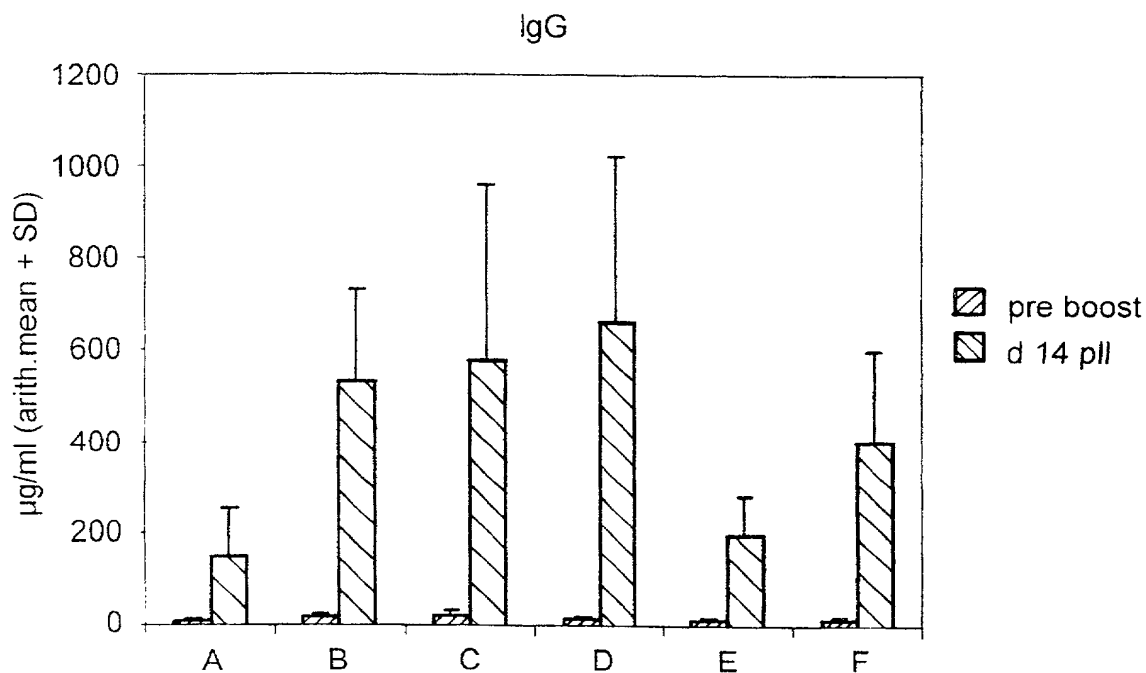


**Fig. 3** Anti-OspA responses in mice (see example 3)

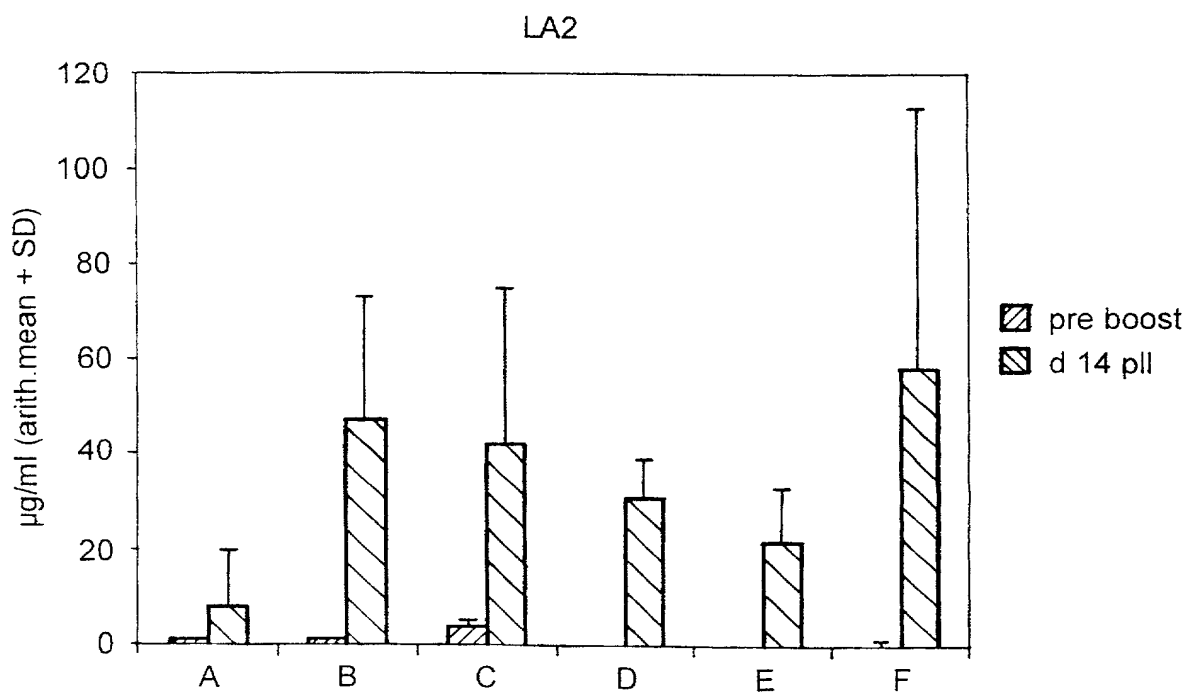


**Fig. 4** LA2 titres in mice (see example 3)

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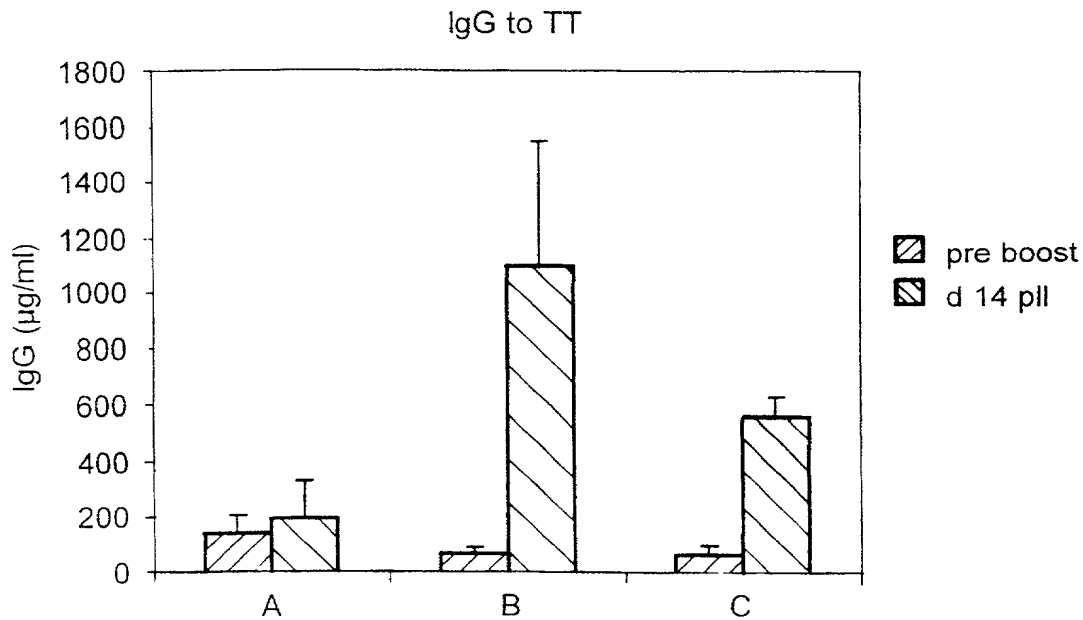


**Fig. 5** Anti-OspA antibody titres (see example 4)

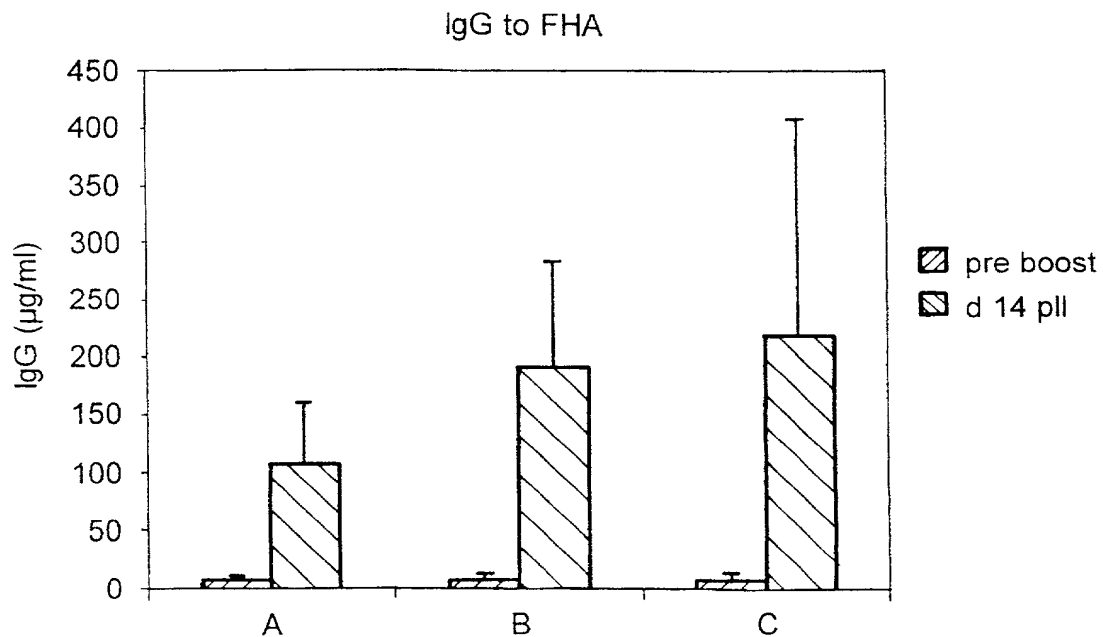


**Fig. 6** LA2 titres in mice (see example 4)

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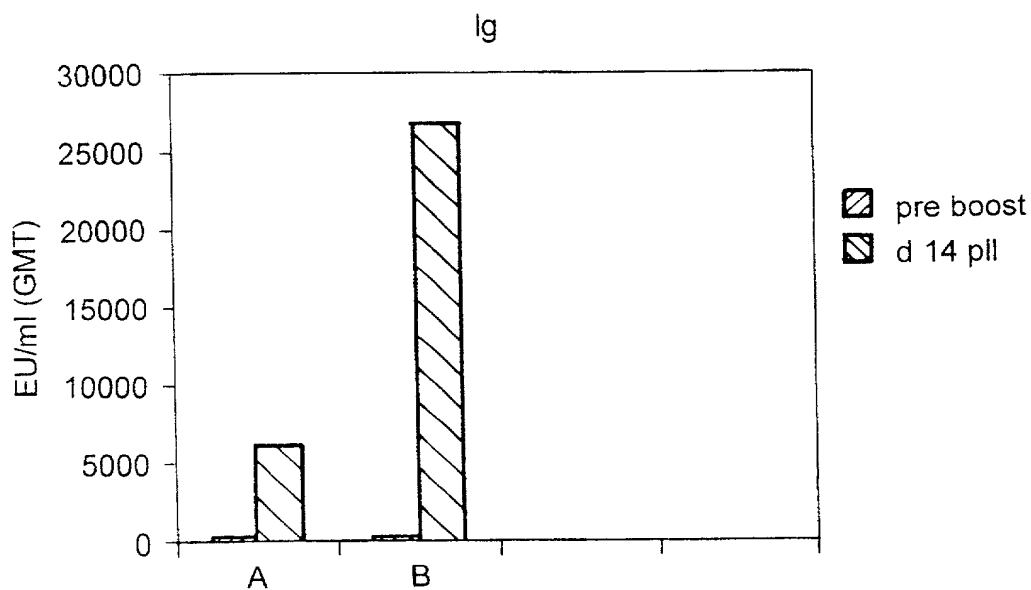


**Fig. 7** Anti-TT immunoglobulin responses as measured by ELISA (see example 5)

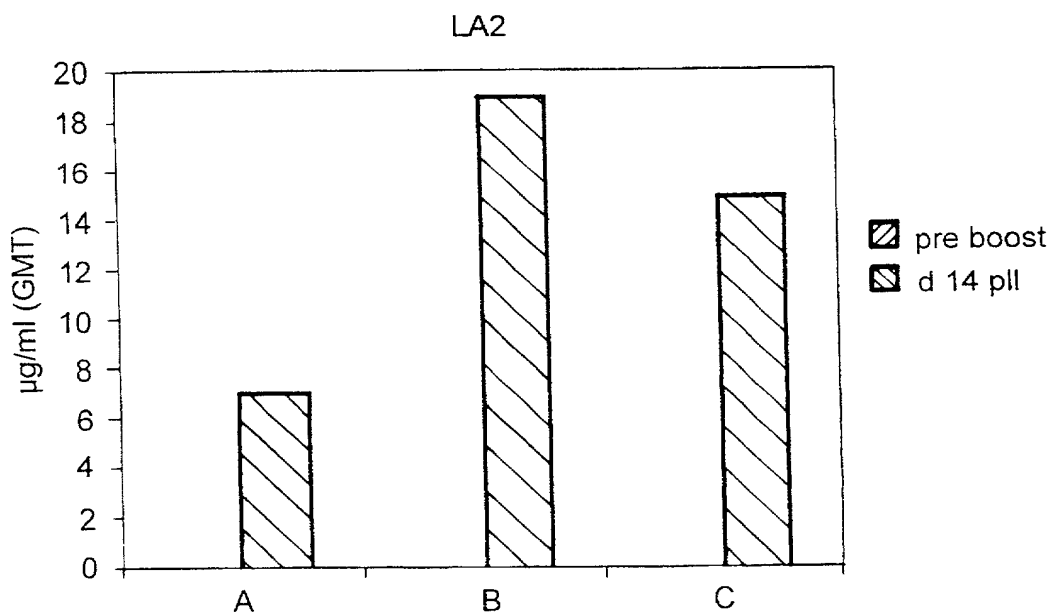


**Fig. 8** Anti FHA immunoglobulin responses as measured by ELISA (see example 5)

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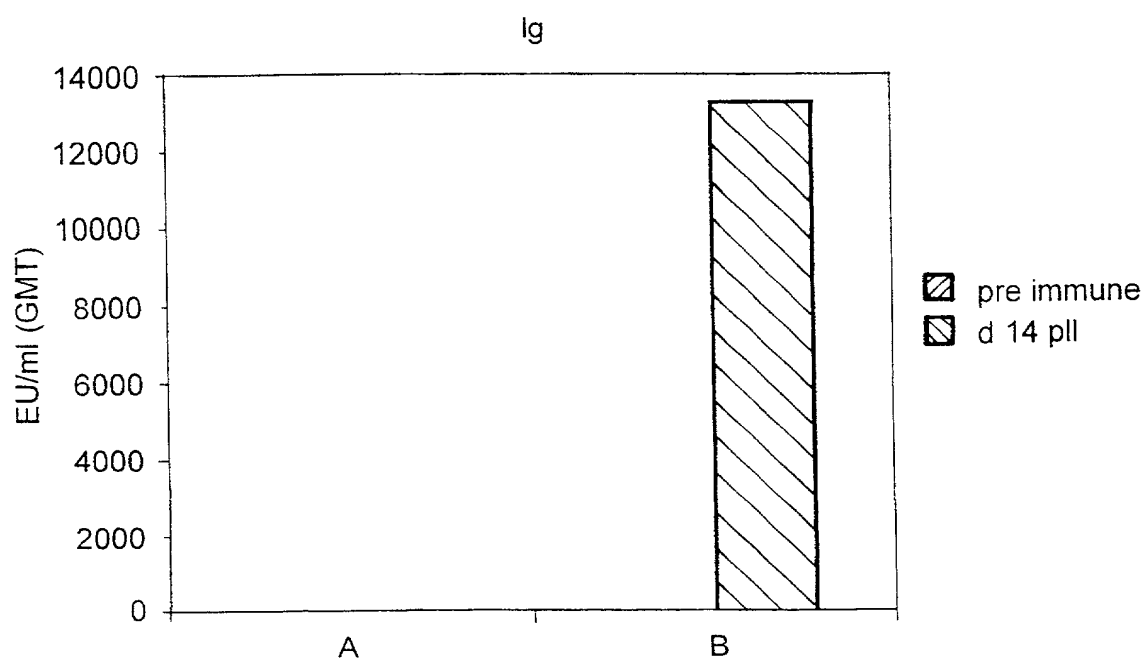


**Fig. 9** Anti-OspA ELISA titres in AGM's (see example 6)



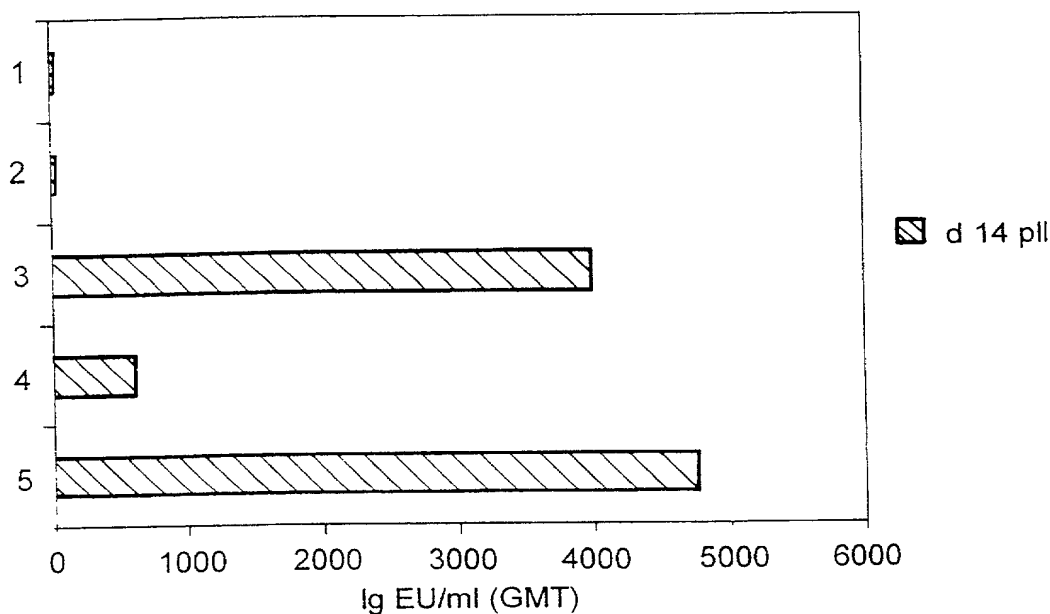
**Fig. 10** LA2 titres in AGM's (see example 6)

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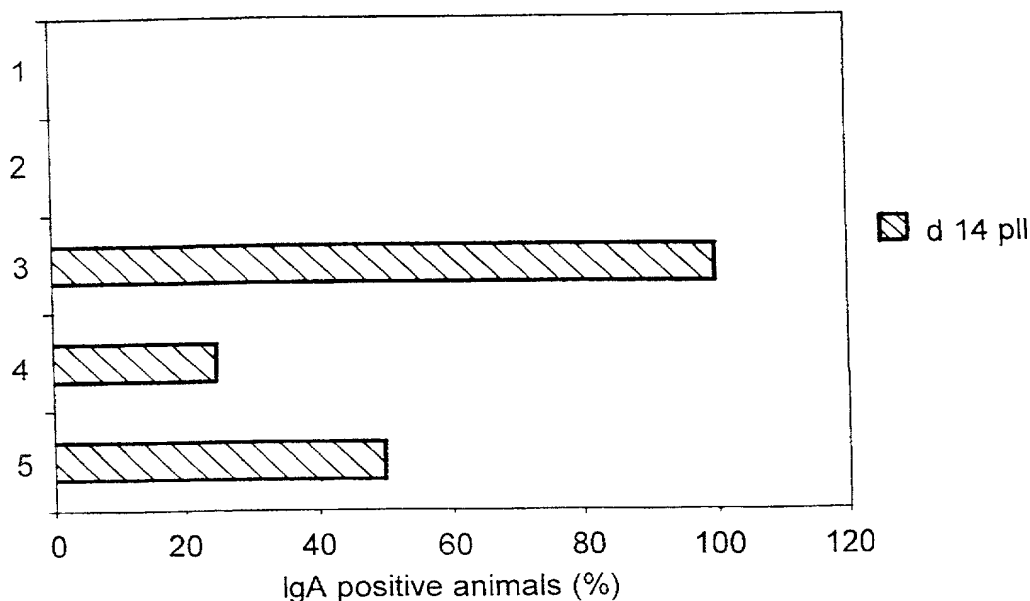


**Fig. 11** Intranasal priming and boosting of AGM's, anti-OspA ELISA responses (see example 7)

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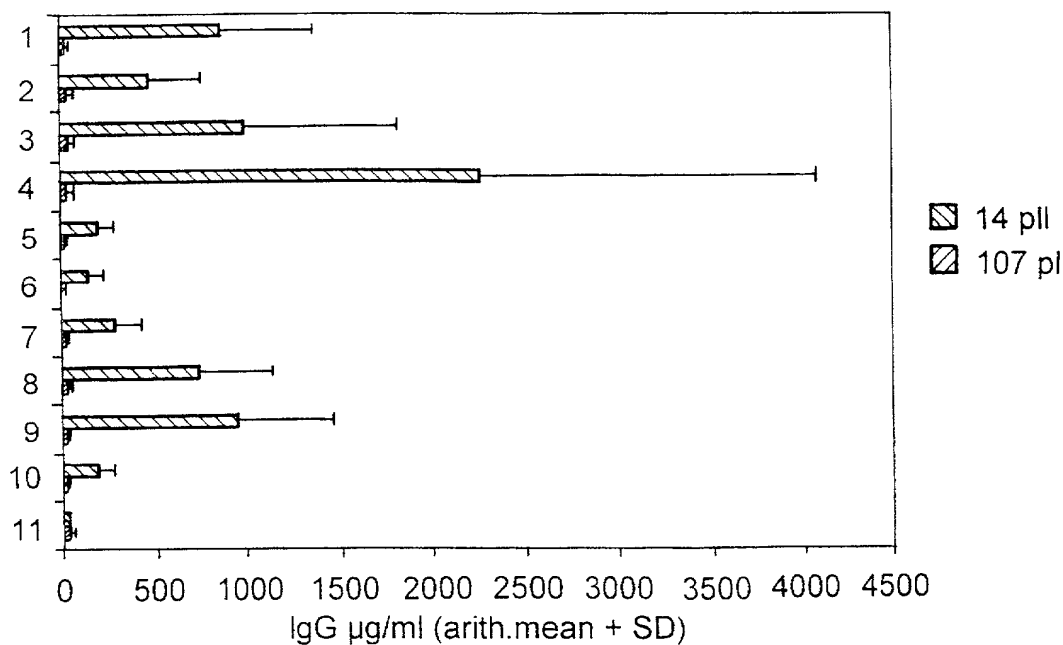
**Fig. 12** Intranasal priming and boosting of African Green Monkeys with POE and CpG vaccine formulations (see example 8)  
Nasal induction of systemic Abs to lipo OspA in monkeys



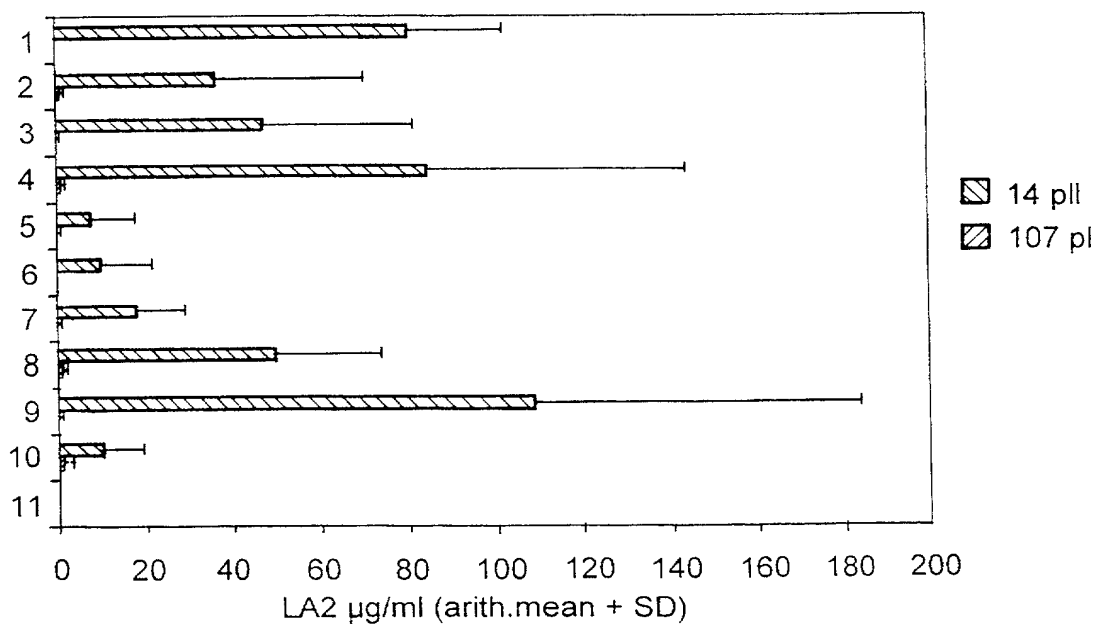
**Fig. 13** Intranasal priming and boosting of African Green Monkeys with POE and CpG vaccine formulations (see example 8)  
Induction of nasal IgA to lipo OsPA in monkeys



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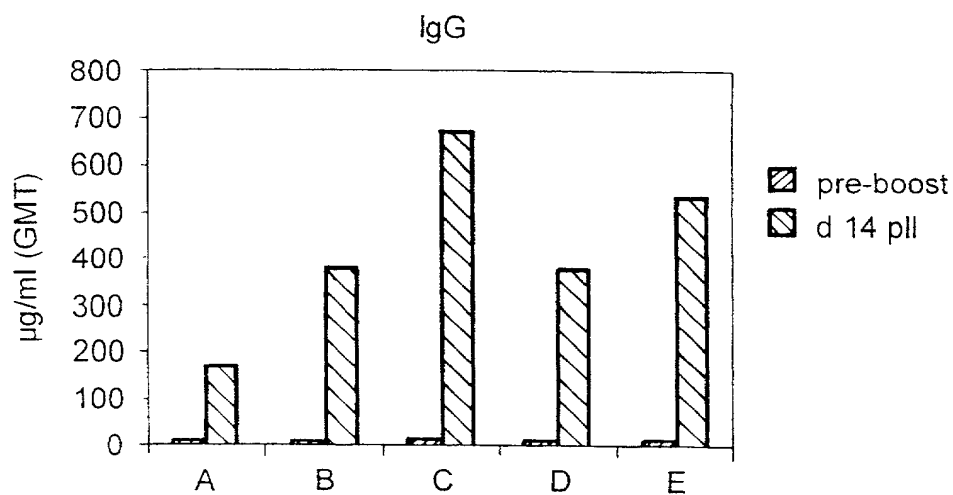


**Fig. 14** Intranasal boosting of mice with POE-9 LE and CpG vaccine formulations (see example 9)  
Nasal boosting of serum IgG

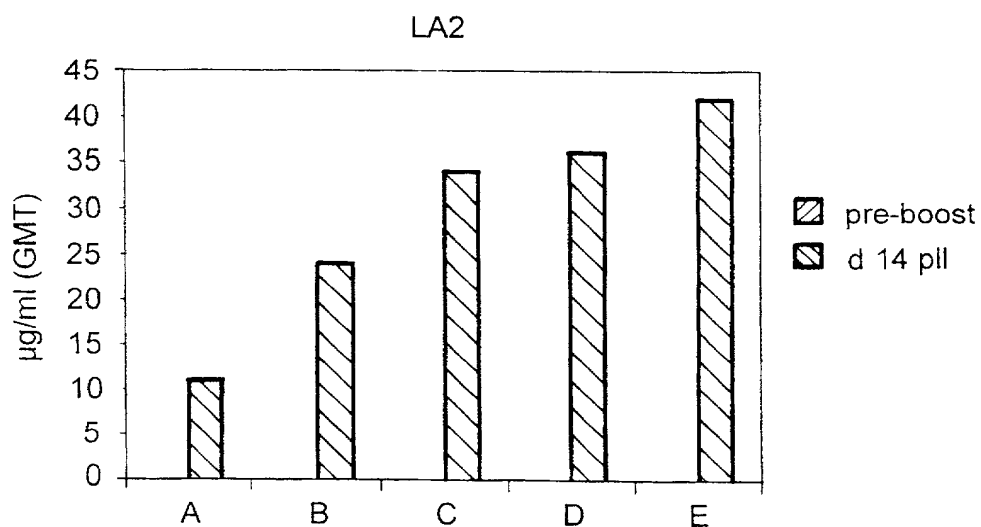


**Fig. 15** Intranasal boosting of mice with POE-9 LE and CpG vaccine formulations (see example 9)  
Nasal boosting of serum LA2 Abs

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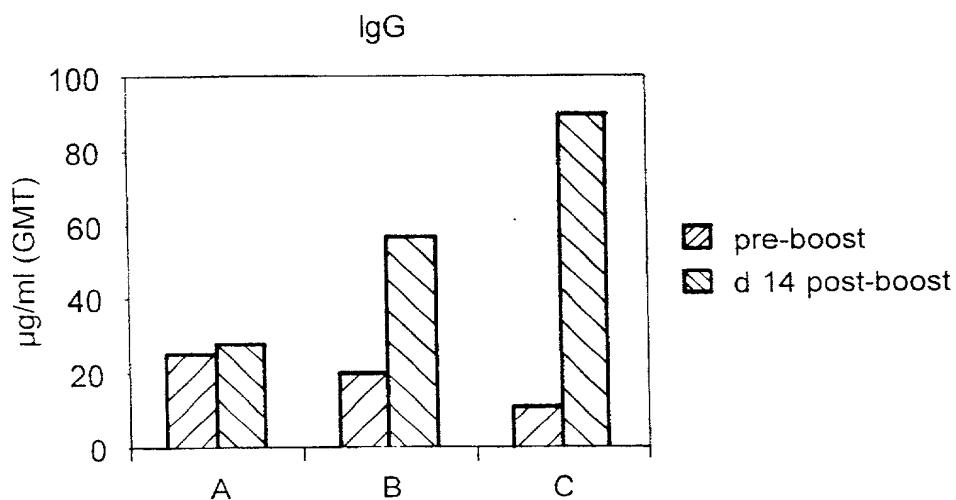


**Fig. 16** Serum IgG responses to Lipo-OspA in mice (see example 10)

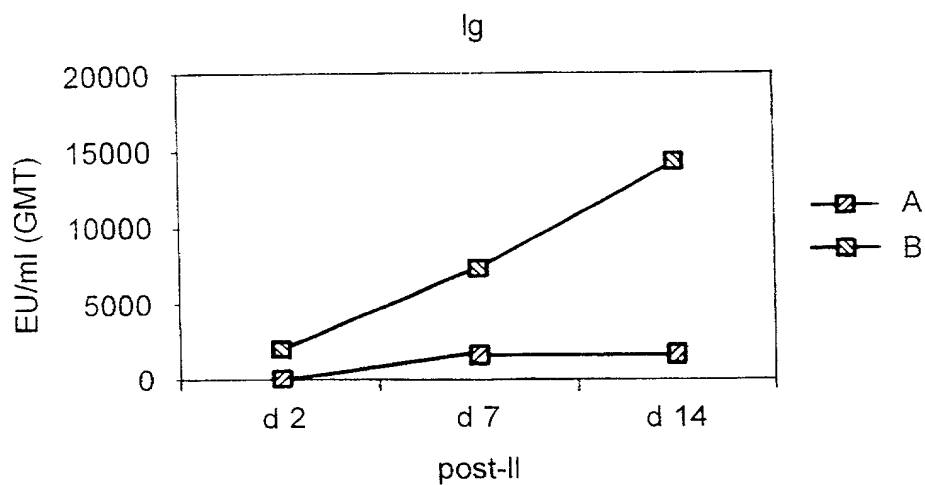


**Fig. 17** Serum LA2 titres in mice (see example 10)

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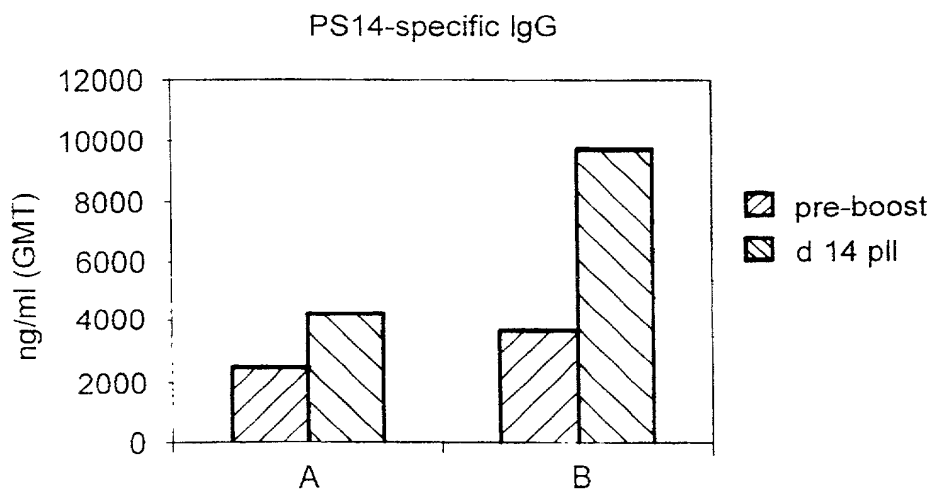


**Fig. 18** Serum IgG response to influenza virus A/Singapore/6/86 in mice (see example 11)

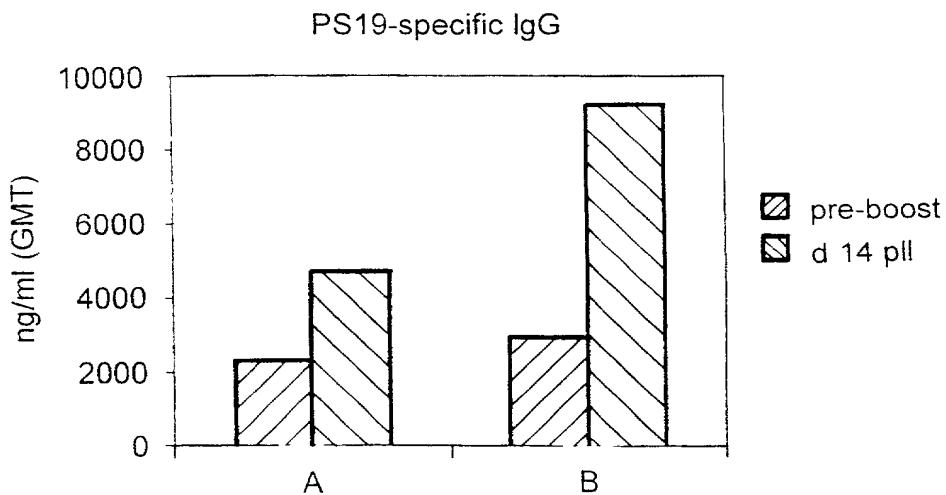


**Fig. 19** Serum Ig Abs to influenza virus A/Beijing/262/95 in Africa Green monkeys (see example 12)

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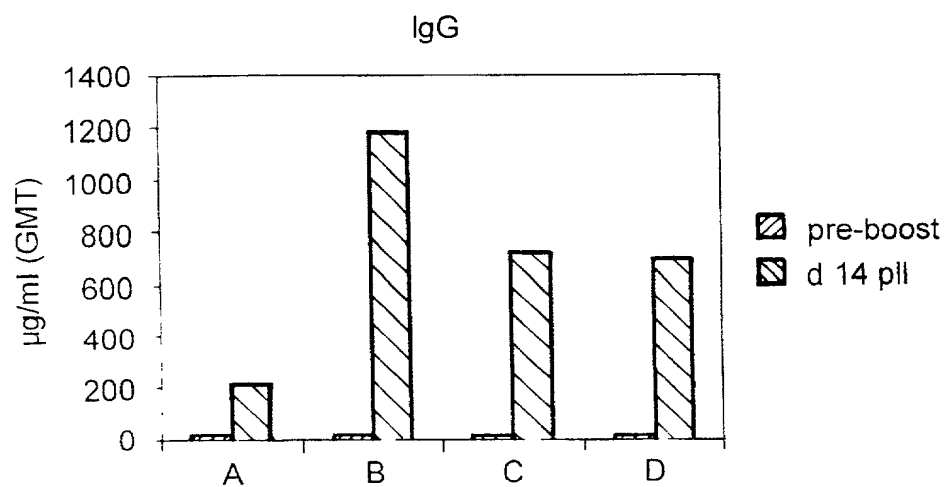


**Fig. 20** Serum IgG response to PS14 in mice  
(see example 13)

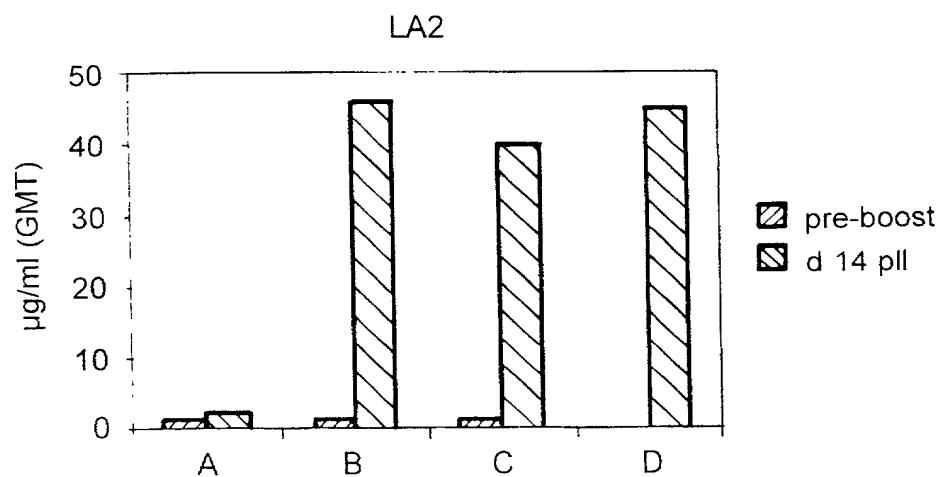


**Fig. 21** Serum IgG response to PS19 in mice  
(see example 13)

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**Fig. 22** Serum IgG responses to Lipo-OspA in mice (see example 14)



**Fig. 23** Serum LA2 titers in mice (see example 14)

Docket No.: B45139

PCT/EP99/02278

## DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

VACCINE

the specification of which (check one)

☐ is attached hereto.

☒ was filed on 29 March 1999 as Serial No. PCT/EP99/02278  
and was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or Inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			
Number	Country	Filing Date	Priority Claimed
9807805.8	Great Britain	09 April 1998	Yes
9820956.2	Great Britain	25 September 1998	Yes

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

Application Number	Filing Date
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I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

Serial No.	Filing Date	Status
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I hereby appoint the practitioners associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to that Customer Number:

Customer Number 20462.

Address all correspondence and telephone calls to Zoltan Kerekes, SmithKline Beecham Corporation, Corporate Intellectual Property-U.S., UW2220, P.O. Box 1539, King of Prussia, Pennsylvania 19406-0939, whose telephone number is 610-270-5024.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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